

Lung function and biomarkers of airway inflammation during and after hospitalization for acute exacerbations of childhood asthma associated with viral respiratory symptoms

Jason S. Debley, MD, MPH; Elizabeth S. Cochrane, BS; Gregory J. Redding, MD; and Edward R. Carter, MD

Department of Pediatrics, Division of Pulmonary Medicine, Seattle Children's Hospital, University of Washington, Seattle, Washington

ARTICLE INFO

Article history:

Received for publication January 11, 2012.

Received in revised form March 21, 2012.

Accepted for publication June 1, 2012.

ABSTRACT

Background: There are limited data assessing relationships between biomarkers of inflammation and lung function after hospitalization for asthma exacerbations in children.

Objective: To assess the associations in asthmatic children among changes in lung function, fraction of exhaled nitric oxide (FENO), and cysteinyl leukotrienes (CysLTs) in exhaled breath condensate (EBC) after hospitalization for acute asthma.

Methods: Spirometry and FENO were measured and EBC collected for CysLT measurement from 40 children during and 1, 2, and 4 weeks after hospitalization for an asthma exacerbation and during a single-study visit for 40 healthy children.

Results: Enrollment FENO and EBC CysLT concentrations were higher in the children with asthma than in healthy individuals (mean FENO, 31.6 vs 7 ppb; $P < .0001$; mean EBC CysLT, 7.9 vs 4.9 ppb; $P = .03$). Among children with asthma, improvement in lung function reached a plateau within 2 weeks after hospital discharge. The EBC CysLT concentrations were not associated with changes in lung function, use of albuterol, or use of inhaled corticosteroids (ICSs). Among asthmatic children enrollment FENO was not associated with changes in lung function during follow-up. However, among children who had an elevated enrollment FENO (≥ 25 ppb), patients who did not use ICSs after hospital discharge had lower end-of-study lung function than those who used ICSs. At 2 and 4 weeks after hospital discharge, FENO was higher among patients who reported albuterol use more than twice weekly and among patients who reported no ICS use.

Conclusion: FENO measured at hospital discharge among children hospitalized with acute asthma may be useful in identifying patients who will respond to ICS therapy.

© 2012 American College of Allergy, Asthma & Immunology. Published by Elsevier Inc. All rights reserved.

Introduction

Asthma, a chronic inflammatory obstructive airway disease, is the most common respiratory disease in children, affecting at least 6.5 million children and causing 7 million physician visits and 200,000 hospitalizations among children annually in the United States.¹ In addition, asthma exacerbations are a major cause of school absenteeism and activity limitation.¹ Systemic corticosteroids and inhaled bronchodilators are widely accepted as cornerstones of effective treatment for acute exacerbations of asthma.² Although recent investigations have improved our understanding of the complex inflammatory pathways involved in acute asthma^{3,4}

and their potential relationships to lung function,⁵ relatively little is known about the expected time course for lung function recovery and resolution of airway inflammation after asthma exacerbations. Specifically, there is a paucity of data regarding how quickly lung function improves or returns to normal, how rapidly airway inflammation resolves, and the optimal dose, delivery route, and duration of corticosteroids with which to treat children after an acute asthma exacerbation. Although lung function in many patients with asthma returns to normal between acute exacerbations, severe exacerbations may be associated with a decline in lung function over time in adults and children.⁶ Thus, a better understanding of airway pathophysiology and the trajectory of improvement in lung function and airway inflammation after asthma exacerbations in children would further inform guidelines for treatment during the recovery phase of an asthma exacerbation.

Methods of directly assessing airway inflammation in patients with asthma (eg, bronchoalveolar lavage, bronchial biopsy, and analysis of induced sputum) are invasive, expensive, or impractical research or diagnostic tools in children. However, 2 proposed non-

Reprints: Jason S. Debley, MD, MPH, Pulmonary Division (A-5937), Seattle Children's Hospital, 4800 Sand Point Way NE, Seattle, WA, 98105; E-mail: jason.debley@seattlechildrens.org.

Disclosures: Authors have nothing to disclose.

Funding Sources: This research was conducted with support from the Investigator-Sponsored Study Program of AstraZeneca, an AstraZeneca Investigator Initiated Study grant, National Heart, Lung, and Blood Institute grant K23HL077626, and NIH Clinical and Translational Science Award grant I UL1 RR025014-01.

invasive biomarkers of airway inflammation in asthma, the fraction of exhaled nitric oxide (FENO)⁷ and cysteinyl leukotrienes (CysLTs) in exhaled breath condensate (EBC),^{8–11} have been shown to be elevated in children with asthma and may have promise in assessing and monitoring airways inflammation in the clinical setting. Concentrations of these exhaled biomarkers of inflammation during and after asthma exacerbations might predict trends in lung function, airway inflammation, and asthma symptoms during the recovery phase after acute asthma exacerbations.

The objectives of our single-center pilot study were to assess changes in lung function and concentrations of FENO and CysLTs in EBC in a convenience sample of children aged 6 to 18 years for 1 month after hospitalization for an acute asthma exacerbation. We hypothesized that after hospitalization for an asthma exacerbation lung function would reach a plateau in improvement between 2 and 4 weeks after hospital discharge and that levels of FENO and CysLTs in EBC would decrease during the follow-up period and would be associated with the degree of airflow obstruction, asthma symptoms, use of albuterol for asthma symptoms, and use of inhaled corticosteroids (ICSs). Finally, we hypothesized that concentrations of FENO and CysLTs in EBC would be higher in patients hospitalized for an asthma exacerbation than in age-matched healthy controls.

Methods

Study participants

Children 6 to 18 years of age with a history of physician-diagnosed asthma of at least 1-year duration, who met National Heart, Lung, and Blood Institute/National Asthma Education and Prevention Program guidelines for the diagnosis of asthma,² who were admitted to Seattle Children's Hospital for treatment of an acute severe asthma exacerbation,¹² and who were able to perform acceptable and reproducible spirometry and FENO measurements were enrolled in the study. In accordance with standard treatment of asthma at our institution, all patients with asthma were treated with systemic corticosteroids (0.6 mg/kg/d of dexamethasone) and bronchodilators during hospitalization. Daily ICSs were prescribed for use after discharge in all patients with asthma. Inpatient and discharge medications and treatment plans were not influenced by any members of the study team but rather were determined by the patient's attending physician. The children with asthma were enrolled and completed initial study measurements within 48 hours of initiation of systemic corticosteroid treatment, with a mean duration of systemic corticosteroid treatment before enrollment measurements of 35.4 hours (range, 19–48 hours). Children 6 to 18 years of age without a history of asthma, reactive airway disease, chronic cough (daily for the month before enrollment), allergic rhinitis, atopic dermatitis, food allergies, or an upper respiratory tract infection or sinusitis within 3 weeks of study entry were recruited as healthy controls. Written consent was obtained from parents of study participants, and written assent was obtained from those 12 years or older. The study was approved by the Seattle Children's Hospital Institutional Review Board.

Study visits

At enrollment pertinent medical history was reviewed (including age of asthma diagnosis, history of hospitalization for asthma, history of skin test positivity or positive radioallergen sorbent testing [RAST] to ≥ 1 aeroallergens, physician-diagnosed and treated eczema, family history of first-degree relative with asthma, environmental tobacco smoke exposure, and upper respiratory tract infection symptoms), FENO was measured, EBC was collected, and lung function testing performed. History of skin test positivity, positive RAST results, and physician-diagnosed and treated eczema were confirmed by review of medical records. Follow-up visits occurred

at 1, 2, and 4 weeks after hospital discharge, during which interval medical history, medication use, and daily symptom diaries were reviewed. In addition, lung function tests, FENO, and EBC collections were repeated. Between study visits, parents of asthmatic patients completed daily symptom and medication use diaries. Diaries were reviewed to assess adherence to prescribed asthma medications.

Lung function and exhaled breath measurements

FENO was measured at an expiratory flow rate of 50 mL/s according to American Thoracic Society guidelines using a NIOX chemiluminescent nitric oxide analyzer (Aerocrine, Solna, Sweden).¹³ Forced expiratory volumes and flows, including forced vital capacity (FVC), forced expiratory volume in 1 second (FEV₁), FEV₁:FVC ratio, and forced expiratory flow between 25% and 75% (FEF_{25%–75%}), were measured according to American Thoracic Society guidelines.¹⁴ At enrollment, spirometry was performed at least 2 hours after a patient's most recent treatment with albuterol. At follow-up visits albuterol was withheld for at least 4 hours before performing spirometry. EBC was collected using the R-Tube system (Respiratory Research, Charlottesville, Virginia) during 20 minutes of tidal breathing while wearing a nose clip. A 1-mL aliquot of EBC was processed using an eicosanoid solid-phase extraction protocol. The EBC samples were acidified to a pH less than 4; then loaded onto a preconditioned Oasis HLB cartridge (Waters, Milford, Massachusetts); then washed with 0.03% formic acid in water, 0.03% formic acid in 10% ethanol, and hexanes; then eluted with 0.2% formic acid in methanol; and then stored for 3 months or less at -80°C until CysLT measurements were performed. Immediately before CysLT assay, samples were evaporated to dryness under argon then resuspended with 0.2 mL of enzyme immunoassay (EIA) buffer solution to achieve a 5-fold sample concentration factor. CysLT concentrations were measured using the Cayman EIA. All samples were analyzed in duplicate. The lower limit of detection on the Cayman EIA standard curve is 7.8 pg/mL. Given that samples were concentrated 5-fold by solid-phase extraction before EIA, our estimated lower limit of detection of CysLTs in EBC was 1.6 pg/mL.

Amylase was measured in undiluted EBC samples with an EnzChek amylase assay kit (E33651) from Invitrogen Molecular Probes (Eugene, Oregon) and found to be undetectable in all samples.

Statistical analysis

We estimated that 40 patients and 40 healthy controls were required to provide more than 90% power to detect a mean difference in FENO or EBC CysLT between the groups of at least 1 SD at the $\alpha = .05$ significance level. Forty patients were estimated to provide at least 80% power to identify FENO as a significant predictor of FEV₁ 4 weeks after hospitalization if FENO accounted for 15% or more of the total variance in FEV₁ (ie, $r^2 = 0.15$).

Unpaired *t* tests or the Mann-Whitney tests for nonnormally distributed data were used to compare enrollment FENO, EBC CysLT concentration, and lung function parameters between asthmatic and healthy individuals. One-way analysis of variance followed by the Tukey multiple comparison posttest were used to compare lung function parameters during follow-up between subsets of asthmatic patients based on the following characteristics: reported consistent (≥ 5 days per week) use of ICSs during follow-up (yes/no), enrollment FENO (normal [<25 ppb] vs elevated [≥ 25 ppb]), and enrollment EBC CysLT concentration (less than median enrollment CysLT vs median enrollment CysLT or greater of asthmatic patients). FENO was compared between those children with asthma using albuterol less than twice weekly vs those using it twice weekly or more using the unpaired *t* test. The Pearson coefficient was used to estimate correlations between biomarkers in exhaled breath (FENO and EBC CysLT) and lung function measures

Table 1
Characteristics of the study participants

Characteristic	Children with asthma completing study (n = 34)	Children with asthma not completing study (n = 6)	Healthy controls (n = 40)
Male, No. (%)	24 (71)	3 (50)	18 (45)
Age at enrollment, mean (SD), y	9.7 (2.9)	10.3 (6.3)	10.8 (3.2)
Race/ethnicity, No. (%)			
White	17 (50)	2 (33)	35 (87)
African American	10 (29)	1 (17)	3 (8)
Asian	1 (3)	1 (17)	1 (3)
Native American	1 (3)	0	0
Hispanic	5 (15)	0	1 (3)
Pacific Islander	0	2 (33)	0
Atopic by skin prick or RAST testing, No. (%)	22 (65)	6 (100)	0
Eczema (physician diagnosed and steroid treated), No. (%)	14 (41)	4 (67)	0
Family history asthma (first-degree relative), No. (%)	23 (68)	5 (83)	5 (13)
Environmental tobacco smoke exposure, No. (%)	9 (26)	3 (50)	6 (15)
History of hospitalization for asthma during the past 12 months, No. (%)	14 (41)	1 (17)	NA
History of intensive care unit stay for asthma, No. (%)	9 (26)	2 (33)	NA
Viral URI symptoms during week prior week before admission, No. (%)	27 (79)	5 (83)	NA
Inhaled corticosteroid use during follow-up (budesonide equivalent dose), No. (%)	18 (53)	NA	NA
200 µg twice daily	9 (26)		
400 µg twice daily	7 (21)		
1 mg once daily	1 (3)		
2 mg once daily	1 (3)		
Systemic steroid treatment (prednisone, 2 mg/kg/d, or dexamethasone, 0.6 mg/kg/d) between hospital admission and enrollment study visit	34 (100)	6 (100)	NA
Enrollment FENO, mean (SD), ppb ^a	31.5 (26.7)	50.8 (45.3)	7.0 (2.4)
Enrollment EBC CysLT, mean (SD), pg/mL ^b	7.9 (7.3)	8.5 (10.2)	4.9 (3.6)
Enrollment FVC, mean (SD), % predicted ^a	88.1 (18.7)	83.3 (19.3)	101.6 (10.0)
Enrollment FEV ₁ , % predicted ^a	76.9 (20.5)	72.8 (16.3)	99.3 (10.8)
Enrollment FEV ₁ :FVC ratio, mean (SD) ^a	76.2 (8)	75.8 (9.6)	90.5 (5.3)
Enrollment FEF _{25%-75%} , mean (SD), % predicted ^a	43.3 (26.7)	45.5 (25.5)	90.7 (19.7)

Abbreviations: CysLT, cysteinyl leukotriene; EBC, exhaled breath condensate; ED, emergency department; FEF_{25%-75%}, forced expiratory flow between 25% and 75%; FENO, fraction of exhaled nitric oxide; FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity; NA, not applicable; RAST, radioallergosorbent testing; URI, upper respiratory tract infection.

^aP < .001 for children with asthma vs healthy controls.

^bP = .03 for children with asthma vs healthy controls.

at enrollment and follow-up visits. FENO and lung function data were determined to be normally distributed by the D'Agostino and Pearson and the Kolmogorov-Smirnov tests. All analyses were conducted using GraphPad Prism 5.0 (GraphPad, LaJolla, California) and SPSS statistical software for Windows, version 11.5 (SPSS Inc, Chicago, Illinois).

Results

Forty children with asthma (mean [SD] age, 9.5 [3.4] years) and 40 healthy children (mean [SD] age, 10.8 [3.2] years) were enrolled. Baseline characteristics of the asthmatic and healthy children are presented in Table 1. The mean enrollment FEV₁, FEV₁:FVC ratio, and FEF_{25%-75%} percent predicted values of the children with asthma were significantly lower than those of the controls. At enrollment the mean FENO and EBC CysLT concentrations of the children with asthma were significantly higher than those in the controls (Fig 1 and eFig 1), but there was a large degree of overlap in EBC CysLT concentrations between the 2 groups. A higher proportion of the children with asthma were boys (71% vs 45%), and more of the controls were white (87% vs 50%). Most of the children with asthma (79%) reported viral upper respiratory tract infection symptoms during the week before hospitalization. Most children had a history of confirmed atopy by skin prick testing or RAST (65%), and 41% of children had a history of physician-diagnosed and treated eczema. Six of the children with asthma were lost to follow-up and did not complete follow-up visits. The age, race/ethnicity, and baseline lung function of asthmatic children completing the study were similar to those lost to follow-up, but a larger proportion of children lost to follow-up were atopic (history of skin test positivity or positive RAST results to ≥1 aeroallergens or history of eczema) and were exposed to environmental tobacco smoke (Table 1). All of the

children with asthma were prescribed an ICS as a daily asthma controller by their managing physicians at hospital discharge, but only 53% of the children reported consistent use of an ICS during the follow-up period.

During the follow-up period, lung function in the asthma group reached a plateau in improvement between 1 and 2 weeks after hospital discharge (Fig 2). FEV₁ percent predicted was significantly higher at the final follow-up visit among patients that reported consistent use of ICSs after hospital discharge (eFig 2). No correlation was found between enrollment FENO or EBC CysLT concentration and enrollment FEV₁ percent predicted (FENO: $r = 0.2$, $P = .30$; EBC CysLT: $r = 0.1$, $P = .70$), or FEF_{25%-75%} percent predicted (FENO:

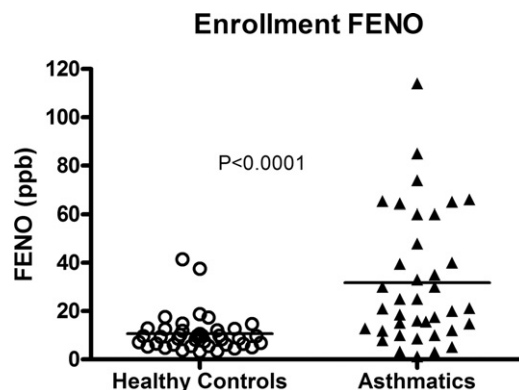


Fig. 1. Concentrations of exhaled nitric oxide (FENO) in parts per billion among healthy controls (open circles) and children hospitalized for an acute exacerbation of asthma (closed triangles).

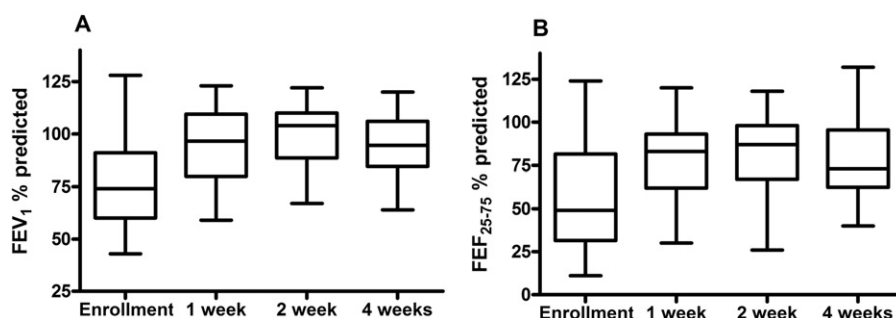


Fig. 2. Lung function trend (forced expiratory volume in 1 second [FEV₁] percent predicted [A] and forced expiratory flow between 25% and 75% [FEF_{25%-75%}] percent predicted [B]) during and after hospitalization for an acute asthma exacerbation.

$r = 0.1$, $P = .60$; EBC CysLT: $r = 0.1$, $P = .90$) (eFig 3). EBC CysLT concentrations decreased between enrollment and 1-week follow-up visits for 8 of the children with asthma, but for all except 1 CysLT concentrations changed little between 1 and 4 weeks after hospital discharge (eFig 4). No correlation was found between enrollment or follow-up CysLT concentrations and lung function, use of albuterol, or reported use of ICSs during the follow-up period, either as an entire group or among the 8 children whose CysLT concentration decreased between enrollment and follow-up visits (data not shown).

FENO declined in most patients between enrollment and the 1-week follow-up visit (eFig 5). Between the 1- and 4-week follow-up visits there was substantial intersubject variability in FENO trends. In the asthma group no correlation was found between enrollment FENO and lung function at the final follow-up visit (FEV₁: $r = 0.2$, $P = .40$; FEF_{25%-75%}: $r = 0.3$, $P = .20$; FEV₁:FVC ratio: $r = 0.2$, $P = .20$).

We divided the children with asthma into 4 categories based on their enrollment FENO (low [<25 ppb] vs high [≥ 25 ppb]) and whether they reported consistent use of ICSs during the follow-up period. For patients who had an enrollment FENO less than 25 ppb, no difference was seen in end-of-study lung function between children who reported consistent ICS use and those who did not. However, patients with an enrollment FENO of 25 ppb or higher who reported that they did not consistently use ICSs during the follow-up period had significantly lower lung function (Table 2 and Fig 3). They were also more likely to report daily use of albuterol (Table 2) than those with an enrollment FENO of 25 ppb or higher who did report consistent ICS use. No significant difference was found among the 4 groups in the duration of systemic corticosteroid treatment before enrollment FENO measurement.

At both 2 and 4 weeks after hospitalization for an acute asthma exacerbation, children reporting use of albuterol greater than twice weekly had significantly higher levels of FENO (eFig 6). FENO decreased in nearly all the children with asthma between hospitalization and the 1-week follow-up visit. However, between 1 and 4 weeks after hospitalization there was substantially more variability in FENO in the children who did not consistently use ICSs during the follow-up period (Fig 4). FENO was significantly higher among children who did not consistently use ICSs at both 2 and 4 weeks after hospitalization (2 weeks: 34.5 vs 12 ppb, $P = .01$; 4 weeks: 34.5 vs 13.8 ppb, $P = .003$; Fig 5). No difference was found in enrollment FENO between children who reported consistent use of ICSs and those who did not (mean FENO, 35.2 ± 8.2 ppb vs 31.9 ± 5.7 ppb; $P = .60$).

Discussion

In a group of children hospitalized for an acute asthma exacerbation, we found that improvement in lung function reached a plateau within 2 weeks after hospital discharge. No correlation was found between enrollment concentrations of FENO or EBC CysLT and enrollment lung function. EBC CysLT concentrations changed little during the 1-month period after hospitalization for an acute asthma exacerbation for most children. Furthermore, EBC CysLT concentrations were not associated with changes in lung function, use of albuterol during follow-up, or consistent use of ICSs during follow-up. Among children with asthma, enrollment FENO was not associated with changes in lung function during follow-up. However, among children with an elevated enrollment FENO (≥ 25 ppb), those who did not consistently use ICS had significantly lower final lung function in the follow-up period than those who consistently used ICSs. Among children with a normal enrollment FENO (<25

Table 2
Clinical outcomes 1 month after hospitalization based on enrollment FENO and ICS use during follow-up

Outcome	FENO <25 ppb and consistent ICS use during follow-up (n = 10)	FENO <25 ppb and inconsistent ICS use during follow-up (n = 8)	FENO ≥ 25 ppb and consistent ICS use during follow-up (n = 8)	FENO ≥ 25 ppb and inconsistent ICS use during follow-up (n = 8)
Daily albuterol use, No. (%)	0	1 (12.5)	0	4 (50) ^a
Missed school, No. (%)	2 (20)	0	1 (12.5)	3 (37.5)
FEV ₁ , mean (SD), % predicted	98.0 (10.5)	91.1 (12.8)	105.0 (5.6)	85.3 (4.7) ^b
FEF _{25%-75%} , mean (SD), % predicted	74.4 (16.1)	82.9 (23.6)	88.1 (6.2)	61.6 (7.5) ^c
Duration of systemic corticosteroid use before enrollment FENO, mean (SD), h	33.2 (8.6)	35.8 (10.6)	37.1 (12.6)	35.9 (7.2)

Abbreviations: FEF_{25%-75%}, forced expiratory flow between 25% and 75%; FENO, fraction of exhaled nitric oxide; FEV₁, forced expiratory volume in 1 second; ICS, inhaled corticosteroid.

^a $P = .03$ compared with enrollment FENO of 25 ppb or higher and consistent ICS use group.

^bAnalysis of variance $P = .02$; Tukey test $P < .05$ for comparison of FENO of 25 ppb or higher and consistent ICS use group vs FENO of 25 ppb or higher and inconsistent ICS use group.

^cAnalysis of variance $P = .02$; Tukey test $P < .05$ for comparison of FENO of 25 ppb or higher and consistent ICS use group vs FENO of 25 ppb or higher and inconsistent ICS use group.

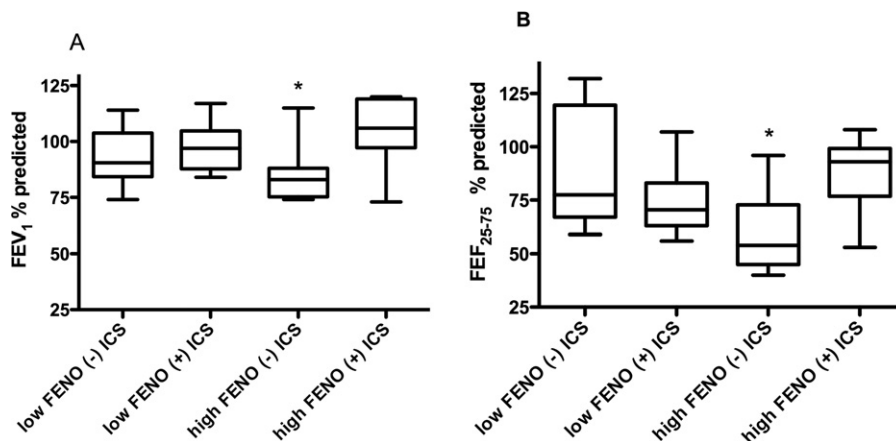


Fig. 3. Lung function 1 month after hospitalization for an acute exacerbation of asthma. FEV₁ indicates forced expiratory volume in 1 second; FEF₂₅₋₇₅, forced expiratory flow between 25% and 75%; ICS, inhaled corticosteroid; low FENO, concentration of exhaled nitric oxide less than 25 parts per billion; high FENO, concentration of exhaled nitric oxide of 25 ppb or greater. Asterisk indicates analysis of variance $P = .02$; Tukey test $P < .05$ for comparison of high FENO with ICS group vs low FENO without ICS group.

ppb), no difference was found in final lung function between children who used ICS consistently and those who did not. At 2 and 4 weeks after hospital discharge FENO was higher in children who reported albuterol use more than twice weekly. Finally, FENO was higher at 2- and 4-week follow-up visits among those who reported that they were not taking an ICS.

Lung function improved in most of our patients after hospitalization for an acute asthma exacerbation, reaching a plateau in improvement 1 to 2 weeks after hospital discharge. As expected, the consistent use of ICS after hospital discharge was associated with better lung function at the final study visit. However, only 53% of patients reported consistent use of ICS after hospital discharge despite the fact that all had been prescribed ICSs as a daily controller by their discharge physician.

Only a few studies have assessed EBC CysLT concentrations during acute asthma exacerbations¹¹ or after allergen challenge.¹⁵ There is also a paucity of data reporting measurements of FENO and lung function in children during hospitalization for acute asthma.^{16–18} Our finding of no correlation between enrollment lung function and enrollment FENO is consistent with data reported by Baptist et al.¹⁶ The mean FENO concentrations in their patients before discharge from the hospital were slightly higher than those in our patients (38.4 vs 31.5 ppb), and the mean enrollment FEV₁ in their patients was also lower (64.4% vs 76.9% predicted). These differences could be explained by their cohort having either more severe asthma or being more acutely ill at the time of enrollment lung function measurements. Our finding of a lack of correlation between FENO and lung function measurements is also consistent with many studies in clinically stable patients with asthma that

showed that FENO is not correlated with, or only weakly correlated with, lung function.^{19–22}

The concentration of CysLTs in EBC has been shown to be higher in patients with asthma compared with healthy adults and children.^{8,9,11,23–25} However, there is considerable range in the concentration of CysLTs in EBC obtained from both asthmatic and healthy individuals, and EBC CysLT concentrations are often below the detection limit of EIAs or liquid chromatography²⁶ in many asthmatic and/or healthy children.^{9,10,27,28} This finding may be in part due to differences in EBC sample processing and storage. We previously reported that concentration of samples using a solid-phase extraction method significantly improves the detection of CysLTs in EBC.²³ We used this method to process EBC samples in this study for CysLT measurement. Our finding that the group mean for EBC CysLT concentrations was higher among our asthmatic compared with our healthy children is consistent with past observations. However, with the exception of 8 asthmatic children in whom EBC CysLT concentrations decreased after hospitalization, for most children CysLT concentrations changed little during the follow-up period. Brussino et al.¹⁵ reported that EBC CysLT concentrations increased after allergen challenge in asthmatic adults.¹⁵ In most of our patients, viral respiratory tract infection was the presumed trigger of their acute exacerbation. Our patients without viral respiratory tract symptoms did not have a known or suspected allergic trigger of their acute exacerbation. Furthermore, EBC CysLT concentrations did not correlate with enrollment or follow-up lung function or with symptoms or medication use during follow-up. Thus, until more sensitive, less variable, and more practical methods of mea-

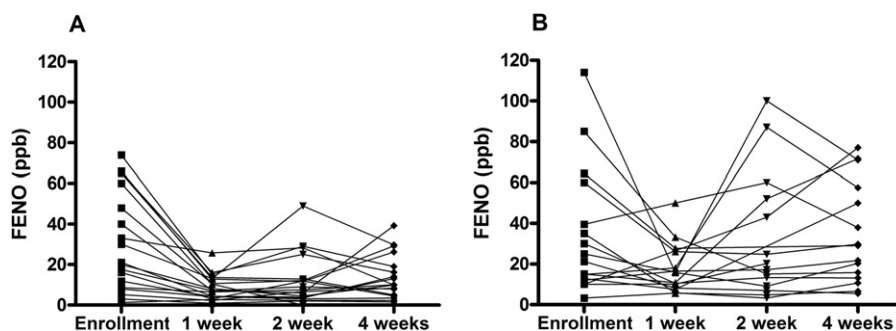


Fig. 4. Exhaled nitric oxide (FENO) concentrations during and after hospitalization for an acute exacerbation of asthma among children who reported consistent use (A) of inhaled corticosteroids (ICSs) and among children who did not use ICSs during follow-up (B).

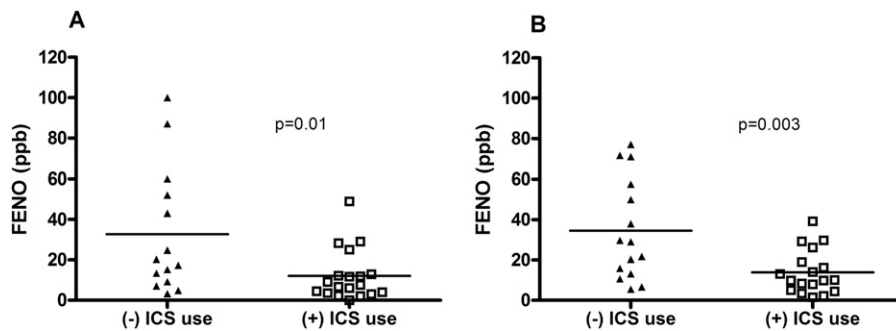


Fig. 5. Exhaled nitric oxide (FENO) concentrations at 2 weeks (A) and 4 weeks (B) after hospitalization for an acute exacerbation of asthma. (–) ICS indicates patients who did not use inhaled corticosteroids during follow-up; (+) ICS, patients who consistently used inhaled corticosteroids during follow-up.

During CysLTs in EBC are readily available, the clinical utility of this biomarker is likely to be limited.

Although FENO concentration at the time of the acute asthma exacerbation was not associated with lung function during the study follow-up period, among the children with asthma who had elevated enrollment FENO (≥ 25 ppb), the consistent use of ICSs resulted in significantly higher lung function at the end of the 1-month study period. Interestingly, among children with a normal enrollment FENO (< 25 ppb), no difference was found in final lung function independent of ICS use during the follow-up period. These results are consistent with observations in adults and children with stable asthma that show that elevated FENO is predictive of a positive response to treatment with ICSs.^{29–31} Of note, during the follow-up period FENO was significantly higher among children who did not consistently use ICSs, suggesting that FENO may also have utility as a marker of adherence with prescribed ICSs. Given that adherence with ICS therapy among asthmatic patients is frequently suboptimal, an objective biomarker, such as FENO, that can predict steroid responsiveness and identify those who are not consistently taking ICSs may be a useful tool to assist clinicians in identifying at risk patients and targeting efforts to improve adherence.

There are several limitations to our study. First, individuals did not complete enrollment lung function, measurement of FENO, and collection of EBC immediately on hospital admission for acute asthma but rather completed initial measurements within 48 hours of initiation of systemic corticosteroid treatment. Because FENO decreases within days of initiation of systemic corticosteroids, we suspect that enrollment FENO concentrations in our patients were lower than what their FENO concentrations would have been if measured at hospital admission or before initiation of systemic steroids. However, we believe the timing of FENO measurement in this study design is more consistent with what could be accomplished in actual clinical situations.

Because patients were recruited during hospitalization for an acute asthma exacerbation, we did not have baseline lung function measurements before hospitalization, so we were unable to determine whether patients truly returned to their baseline lung function. In addition, because we did not perform postbronchodilator spirometry or methacholine challenge in our patients we were unable to assess associations between FENO concentrations and bronchodilator responsiveness or airway hyperreactivity. Although most of our children with asthma reported upper respiratory tract infection symptoms in the week preceding hospitalization, we did not perform an objective confirmation of viral infection. Finally, although matched for age, our healthy control group was not well matched to our asthma group with regard to sex or ethnicity, variables that may influence levels of exhaled biomarkers.

In conclusion, we have shown that in children hospitalized with acute asthma exacerbations lung function improvement plateaus

between 1 and 2 weeks after hospitalization and concentrations of CysLT in EBC do not correlate with lung function during or after hospitalization. However, in patients with elevated FENO during hospitalization for acute asthma, those who reported consistent use of ICSs after hospitalization had significantly better lung function and used less albuterol 1 month after hospital discharge than those who did not use ICSs. Our findings suggest that FENO measurement at hospital discharge in children hospitalized for acute asthma may be helpful in predicting subsequent response to maintenance ICS.

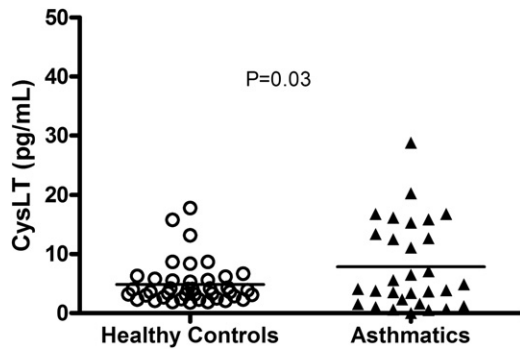
Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.anai.2012.06.004.

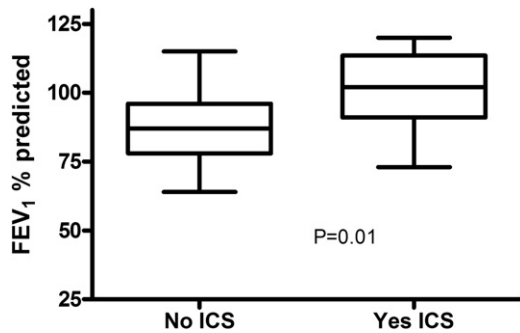
References

- [1] Bloom B, Cohen RA, Freeman G. Summary health statistics for U.S. children: National Health Interview Survey, 2007. *Vital Health Stat* 10. 2009;239:1–80.
- [2] National Heart, Lung, and Blood Institute, National Institutes of Health. National Asthma Education and Prevention Program Expert Panel Report 3 (EPR3): Guidelines for the Diagnosis and Management of Asthma. <http://www.nhlbi.nih.gov/guidelines/asthma/asthgdln.htm>. Accessed January 11, 2012.
- [3] Holt PG, Strickland DH. Interactions between innate and adaptive immunity in asthma pathogenesis: new perspectives from studies on acute exacerbations. *J Allergy Clin Immunol*. 2010;125:963–973.
- [4] Subrata LS, Bizzintino J, Mamessier E, et al. Interactions between innate antiviral and atopic immunoinflammatory pathways precipitate and sustain asthma exacerbations in children. *J Immunol*. 2009;183:2793–2800.
- [5] Bosco A, Ehteshami S, Stern DA, Martinez FD. Decreased activation of inflammatory networks during acute asthma exacerbations is associated with chronic airflow obstruction. *Mucosal Immunol*. 2010;3:399–409.
- [6] O'Byrne PM, Pedersen S, Lamm CJ, Tan WC, Busse WW. Severe exacerbations and decline in lung function in asthma. *Am J Respir Crit Care Med*. 2009;179:19–24.
- [7] Kercsmar C. Exhaled nitric oxide in the diagnosis and management of childhood asthma. *Ther Adv Respir Dis*. 2010;4:71–82.
- [8] Csoma Z, Kharitonov SA, Balint B, Bush A, Wilson NM, Barnes PJ. Increased leukotrienes in exhaled breath condensate in childhood asthma. *Am J Respir Crit Care Med*. 2002;166:1345–1349.
- [9] Zanconato S, Carraro S, Corradi M, et al. Leukotrienes and 8-isoprostane in exhaled breath condensate of children with stable and unstable asthma. *J Allergy Clin Immunol*. 2004;113:257–263.
- [10] Carraro S, Corradi M, Zanconato S, et al. Exhaled breath condensate cysteinyl leukotrienes are increased in children with exercise-induced bronchoconstriction. *J Allergy Clin Immunol*. 2005;115:764–770.
- [11] Baraldi E, Carraro S, Alinovi R, et al. Cysteinyl leukotrienes and 8-isoprostane in exhaled breath condensate of children with asthma exacerbations. *Thorax*. 2003;58:505–509.
- [12] Reddel HK, Taylor DR, Bateman ED, et al. American Thoracic Society/European Respiratory Society Task Force on Asthma C, Exacerbations. An official American Thoracic Society/European Respiratory Society statement: asthma control and exacerbations: standardizing endpoints for clinical asthma trials and clinical practice. *Am J Respir Crit Care Med*. 2009;180:59–99.
- [13] American Thoracic Society, European Respiratory Society. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. *Am J Respir Crit Care Med*. 2005;171:912–930.
- [14] Gardner RM, Hankinson JL. Standardization of spirometry: 1987 ATS update (American Thoracic Society). *J Occup Med*. 1988;30:272–273.

- [15] Brussino L, Badiu I, Sciascia S, et al. Oxidative stress and airway inflammation after allergen challenge evaluated by exhaled breath condensate analysis. *Clin Exp Allergy*. 2010;40:1642–1647.
- [16] Baptist AP, Khan FI, Wang Y, Ager J. Exhaled nitric oxide measurements in hospitalized children with asthma. *J Asthma*. 2008;45:670–674.
- [17] Baptist AP, Sengupta R, Pranathiageswaran S, Wang Y, Ager J. Evaluation of exhaled nitric oxide measurements in the emergency department for patients with acute asthma. *Ann Allergy Asthma Immunol*. 2008;100:415–419.
- [18] Baptist AP, Shah B, Wang Y, Ager J, Badr MS. Exhaled nitric oxide levels during treatment in patients hospitalized with asthma. *Allergy Asthma Proc*. 2008;29:171–176.
- [19] Strunk RC, Szeffler SJ, Phillips BR, et al. Childhood Asthma Research and Education Network of the National Heart, Lung, and Blood Institute. Relationship of exhaled nitric oxide to clinical and inflammatory markers of persistent asthma in children. *J Allergy Clin Immunol*. 2003;112:883–892.
- [20] Mappa L, Cardinale F, Camodeca R, et al. Exhaled nitric oxide and air trapping correlation in asthmatic children. *Allergy*. 2005;60:1436–1439.
- [21] Spergel JM, Fogg MI, Bokszczanin-Knosala A. Correlation of exhaled nitric oxide, spirometry and asthma symptoms. *J Asthma*. 2005;42:879–883.
- [22] Sippel JM, Holden WE, Tilles SA, et al. Exhaled nitric oxide levels correlate with measures of disease control in asthma. *J Allergy Clin Immunol*. 2000;106:645–650.
- [23] Debley JS, Hallstrand TS, Monge T, Ohanian A, Redding GJ, Zimmerman J. Methods to improve measurement of cysteinyl leukotrienes in exhaled breath condensate from subjects with asthma and healthy controls. *J Allergy Clin Immunol*. 2007;120:1216–1217.
- [24] Cap P, Pehal F, Chladek J, Maly M. Analysis of exhaled leukotrienes in nonasthmatic adult patients with seasonal allergic rhinitis. *Allergy*. 2005;60:171–176.
- [25] Buccioni E, Csoma Z, Allegra L, Chung KF, Barnes PJ, Kharitonov SA. Adenosine 5'-monophosphate increases levels of leukotrienes in breath condensate in asthma. *Respir Med*. 2004;98:651–655.
- [26] Kita Y, Takahashi T, Uozumi N, Shimizu T. A multiplex quantitation method for eicosanoids and platelet-activating factor using column-switching reversed-phase liquid chromatography-tandem mass spectrometry. *Anal Biochem*. 2005;342:134–143.
- [27] Biernacki WA, Kharitonov SA, Biernacka HM, Barnes PJ. Effect of montelukast on exhaled leukotrienes and quality of life in asthmatic patients. *Chest*. 2005;128:1958–1963.
- [28] Sandrini A, Ferreira IM, Gutierrez C, Jardim JR, Zamel N, Chapman KR. Effect of montelukast on exhaled nitric oxide and nonvolatile markers of inflammation in mild asthma. *Chest*. 2003;124:1334–1340.
- [29] Smith AD, Cowan JO, Brassett KP, et al. Exhaled nitric oxide: a predictor of steroid response. *Am J Respir Crit Care Med*. 2005;172:453–459.
- [30] Knuffman JE, Sorkness CA, Lemanske RF Jr, et al; Childhood Asthma Research Network of the National Heart Lung and Blood Institute. Phenotypic predictors of long-term response to inhaled corticosteroid and leukotriene modifier therapies in pediatric asthma. *J Allergy Clin Immunol*. 2009;123:411–416.
- [31] Zeiger RS, Szeffler SJ, Phillips BR, et al. Response profiles to fluticasone and montelukast in mild-to-moderate persistent childhood asthma. *J Allergy Clin Immunol*. 2006;117:45–52.

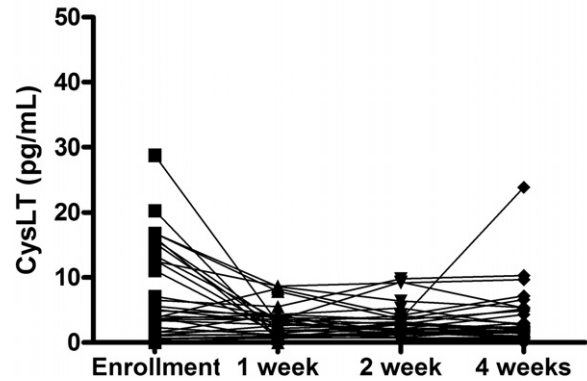


eFig. 1. Concentrations of cysteinyl leukotrienes (CysLTs) in exhaled breath condensate among healthy controls (open circles) and children hospitalized for an acute exacerbation of asthma (closed triangles).

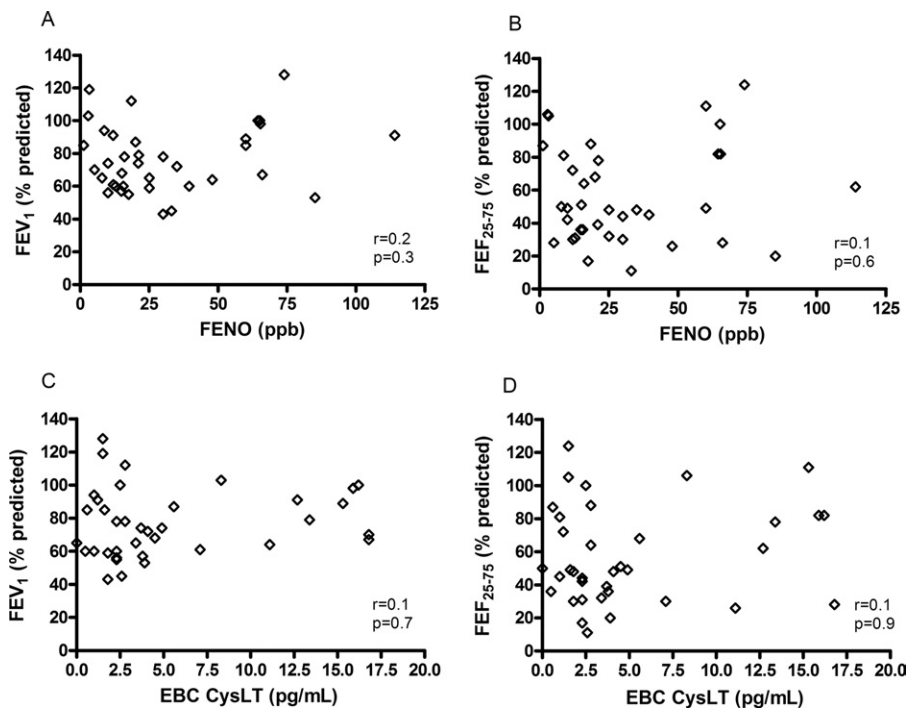


eFig. 2. Forced expiratory volume in 1 second (FEV_1) percent predicted in children with asthma 1 month after hospitalization for an acute exacerbation of asthma. ICS indicates inhaled corticosteroid.

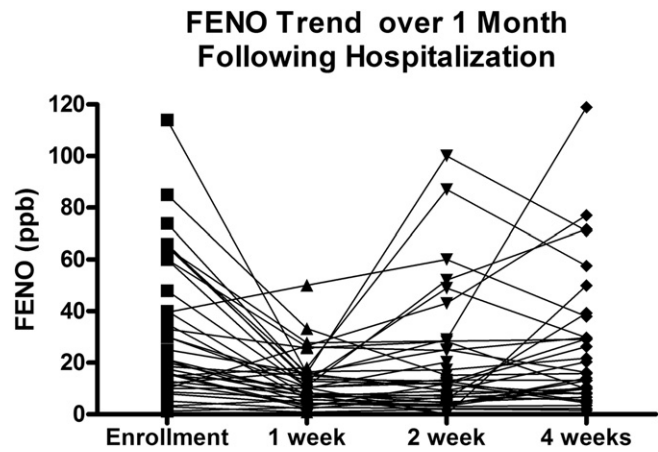
EBC CysLT Concentrations over 1 Month Following Hospitalization



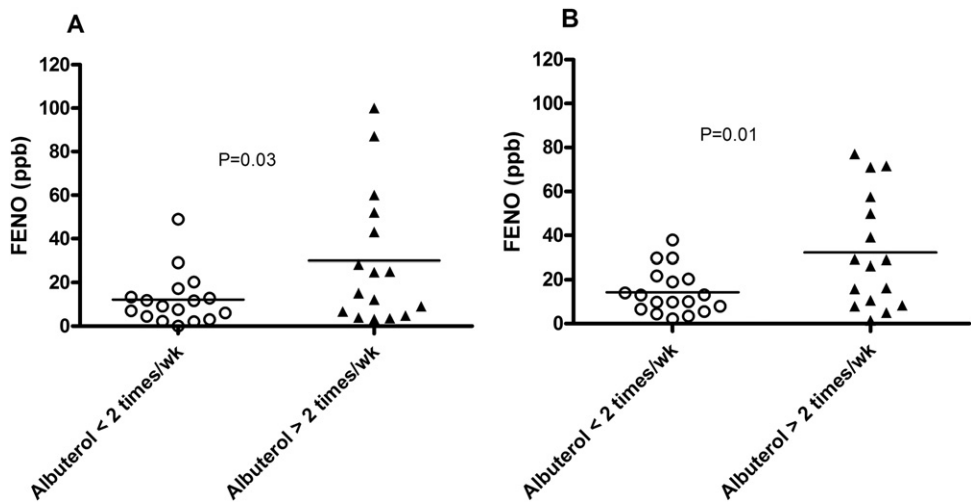
eFig. 4. Concentrations of cysteinyl leukotrienes (CysLTs) in exhaled breath condensate (EBC) during and after hospitalization for an acute exacerbation of asthma.



eFig. 3. Correlation between enrollment fraction of exhaled nitric oxide (FENO) and enrollment lung function (forced expiratory volume in 1 second [FEV_1] percent predicted [A] and forced expiratory flow between 25% and 75% [$FEF_{25-75\%}$] percent predicted [B]) among children with asthma. Correlation between enrollment cysteinyl leukotrienes (CysLTs) in exhaled breath condensate (EBC) and enrollment lung (FEV_1 percent predicted [C] and $FEF_{25-75\%}$ percent predicted [D]) among children with asthma.



eFig. 5. Concentrations of fraction of exhaled nitric oxide (FENO) during and after hospitalization for an acute exacerbation of asthma.



eFig. 6. Enrollment concentration of fraction of exhaled nitric oxide (FENO) and subsequent use of albuterol 2 weeks (A) and 4 weeks (B) after hospitalization for an acute exacerbation of asthma.