Published in final edited form as:

Ann Allergy Asthma Immunol. 2012 August ; 109(2): 114–120. doi:10.1016/j.anai.2012.06.004.

Lung Function and Biomarkers of Airway Inflammation During and Following 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, WA

Keywords

exhaled nitric oxide; FE_{NO}; exhaled breath condensate; cysteinyl leukotrienes; asthma; lung function; exacerbation; hospitalization

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 U.S.¹ In addition, asthma exacerbations are a major cause of school absenteeism and activity limitation.¹ The use of 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 function⁵, relatively little is known about the expected time course for lung function recovery and resolution of airway inflammation following 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 following an acute asthma exacerbation. Although lung function in many patients with asthma returns to normal in 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 following 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, e.g. bronchoalveolar lavage, bronchial biopsy, and analysis of induced sputum, are either invasive, expensive, or impractical research or diagnostic tools in children. However, two

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

Corresponding Author: Jason S. Debley, MD, MPH, ADDRESS: Pulmonary Division (A-5937), Seattle Children's Hospital, 4800 Sand Point Way NE, Seattle, WA, 98105; Telephone: 206-987-2174; FAX: 206-987-2639; jason.debley@seattlechildrens.org.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Conflicts of interest: None of the authors have any conflicts of interest.

proposed non-invasive biomarkers of airway inflammation in asthma, the fraction of exhaled nitric oxide (FENO) ⁷ and cysteinyl leukotrienes (CysLTs) in breath condensate (EBC) ^{8–11}, have been shown to be elevated in children with asthma and may have promise in assessing/monitoring airways inflammation in the clinical setting. Concentrations of these exhaled biomarkers of inflammation during and following asthma exacerbations might predict trends in lung function, airway inflammation, and asthma symptoms during the recovery phase following 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 6–18 years over one month following hospitalization for an acute asthma exacerbation. We hypothesized that following hospitalization for an asthma exacerbation lung function would reach a plateau in improvement between 2–4 weeks following 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 the use of inhaled corticosteroids. Finally, we hypothesized that concentrations of FENO and CysLTs in EBC would be higher in subjects hospitalized for an asthma exacerbation than in age-matched healthy control subjects.

METHODS

Subjects

Children 6–18 years of age with a history of physician-diagnosed asthma of at least 1 year duration, and who met NHLBI/NAEPP guidelines for the diagnosis of asthma², who were admitted to Seattle Children's Hospital for treatment of an acute severe asthma exacerbation¹² and were able to perform acceptable and reproducible spirometry and FENO measurements were enrolled. In accordance with standard treatment of asthma at our institution all patients with asthma were treated with systemic corticosteroids (0.6 mg/kg/day of dexamethasone) and bronchodilators during hospitalization. Daily inhaled corticosteroids (ICS) were prescribed for use following 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 prior to enrollment measurements of 35.4 hours (range 19 – 48hrs.). Children 6–18 years of age without a history of asthma, reactive airway disease, chronic cough (daily over the month prior to enrollment), allergic rhinitis, atopic dermatitis, food allergies, or an upper respiratory infection or sinusitis within 3 weeks of study entry, were recruited as healthy controls. Written consent was obtained from parents of subjects, and written assent was obtained from age 12 years. 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, past history of hospitalization for asthma, history of skin test positivity or positive RAST testing to 1 aeroallergen, 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 testing, and physician-diagnosed and treated eczema were confirmed by review of medical records. Follow-up visits occurred at 1 week, 2 weeks, and 4 weeks following hospital discharge, during which interval medical history, medication use, and daily symptom diaries were reviewed; and

lung function tests, FENO and EBC collections were repeated. Between study visits, parents of asthmatic subjects 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/sec according to American Thoracic Society (ATS) guidelines using a NIOX chemiluminescent nitric oxide analyzer (Aerocrine®, Sweden).¹³ Forced expiratory volumes and flows (FVC, FEV₁, FEV₁/FVC ratio, and FEF₂₅₋₇₅) were measured according to ATS guidelines.¹⁴ At enrollment, spirometry was performed at least 2 hours following a subject'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, VA) 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 (SPE) protocol. EBC samples were acidified to a pH <4, then loaded onto a pre-conditioned Oasis® HLB cartridge (Waters, Milford, MA), then washed with 0.03% formic acid in water, 0.03% formic acid in 10% ethanol, and hexanes, and then eluted with 0.2% formic acid in methanol, then stored for 3 months at -80°C until CysLT measurements were performed. Immediately prior to CysLT assay, samples were evaporated to dryness under argon then re-suspended with 0.2 mL of 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 SPE prior to 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, OR) and found to be undetectable in all samples.

Statistical Analysis

We estimated that 40 patients and 40 healthy subjects were required to provide >90% power to detect a mean difference in FENO or EBC CysLT between the groups of at least one standard deviation at the $\alpha=0.05$ significance level. Forty patients were estimated to provide at least 80% power to identify FENO as a significant predictor of FEV₁ four weeks following hospitalization if FENO accounted for 15% of the total variance in FEV₁ (i.e. $r^2=0.15$). Unpaired t-tests, or the Mann-Whitney test for non-normally distributed data, were used to compare enrollment FENO, EBC CysLT concentration, and lung function parameters between asthmatic and healthy subjects. One-way ANOVA followed by the Tukey's multiple comparison post test were used to compare lung function parameters during follow-up between subsets of asthmatic patients based upon the following characteristics: reported consistent (>5 days/week) use of inhaled corticosteroids (ICS) during follow-up (yes/no), enrollment FENO (normal: <25ppb vs. elevated: ≥25ppb), and enrollment EBC CysLT concentration (< median enrollment CysLT vs. ≥ median enrollment CysLT of asthmatic subjects). FENO was compared between those children with asthma using albuterol < twice weekly vs. those using it ≥ twice weekly 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 at enrollment and follow-up visits. FENO and lung function data was determined to be normally distributed by the D'Agostino and Pearson as well as Kolmogorov-Smirnov tests. All analyses were conducted using GraphPad® Prism 5.0 (LaJolla, CA, USA) and SPSS for Windows® version 11.5 (Chicago, IL, USA).

RESULTS

Forty children with asthma (mean age, 9.5 years [$SD \pm 3.4$]) and 40 healthy children (mean age of 10.8 [$SD \pm 3.2$]) were enrolled. Baseline characteristics of the asthmatic and healthy children are presented in Table I. The mean enrollment FEV_1 , FEV_1/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 (Figures 1 and E1), 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 Caucasian (87% vs. 50%). Most of the children with asthma (79%) reported viral upper respiratory tract infection symptoms during the week prior to hospitalization. A majority of children had a history of confirmed atopy by skin prick testing or RAST testing (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 testing to 1 aeroallergen, or history of eczema) and were exposed to environmental tobacco smoke (Table I). All of the children with asthma were prescribed ICS as a daily asthma controller by their managing physicians at hospital discharge, but only 53% of subjects reported consistent use of 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 following hospital discharge (Figure 2). FEV_1 % predicted was significantly higher at the final follow-up visit among patients that reported consistent use of inhaled steroids following hospital discharge (Figure E2). There was no correlation between enrollment FENO or EBC CysLT concentration and enrollment FEV_1 % predicted (FENO: $r = 0.2$, $p=0.3$; EBC CysLT: $r=0.1$, $p=0.7$) or FEF_{25-75} % predicted (FENO: $r = 0.1$, $p=0.6$; EBC CysLT: $r=0.1$, $p=0.9$) (Figure E3). EBC CysLT concentrations declined between enrollment and 1 week follow-up visits for 8 of the children with asthma, but for all except one CysLT concentrations changed little between 1 and 4 weeks following hospital discharge (Figure E4). There was no correlation between enrollment or follow-up CysLT concentrations and lung function, use of albuterol, or reported use of ICS during the follow-up period, either as an entire group or among the 8 subjects whose CysLT concentration declined between enrollment and follow-up visits (data not shown).

FENO declined in most patients between enrollment and the 1 week follow-up visit (Figure E5). Between the 1 and 4 week follow-up visits there was substantial inter-subject variability in FENO trends. In the asthma group there was no correlation between enrollment FENO and lung function at the final follow-up visit (FEV_1 : $r=0.2$, $p=0.4$; FEF_{25-75} : $r=0.3$, $p=0.2$; FEV_1/FVC : $r=0.2$, $p=0.2$).

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 ICS during the follow-up period. For patients who had an enrollment FENO < 25 ppb, there was no difference in end-of study lung function between children who reported consistent ICS use and those who did not. However, patients with an enrollment FENO ≥ 25 ppb who reported that they did not consistently use ICS during the follow-up period had significantly lower lung function (Table II and Figure 3). They were also more likely to report daily use of albuterol (Table II) than subjects with an enrollment FENO ≥ 25 ppb who did report consistent ICS use. There was no significant difference between the 4 groups in the duration of systemic corticosteroid treatment prior to enrollment FENO measurement.

At both 2 and 4 weeks following hospitalization for an acute asthma exacerbation, children reporting use of albuterol greater than twice weekly had significantly higher levels of FENO (Figure E6). FENO decreased in nearly all the children with asthma between hospitalization and the 1 week follow-up visit. However, between 1 and 4 weeks following hospitalization there was substantially more variability in FENO in the children who did not consistently use ICS during the follow-up period (Figure 4). FENO was significantly higher among subjects who did not consistently use ICS at both 2 and 4 weeks following hospitalization (2 weeks: 34.5 vs. 12 ppb, $p=0.01$; 4 weeks: 34.5 vs. 13.8 ppb, $p=0.003$; Figure 5). There was no difference in enrollment FENO between children who reported consistent use of ICS and those who did not (mean FENO 35.2 $\pm$ 8.2 ppb vs. 31.9 $\pm$ 5.7 ppb; $p=0.6$).

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 following hospital discharge. There was no correlation between enrollment concentrations of FENO or EBC CysLT and enrollment lung function. EBC CysLT concentrations changed little over the one month period following hospitalization for an acute asthma exacerbation for the majority of subjects. Furthermore, EBC CysLT concentrations were not associated with changes in lung function, use of albuterol during follow-up, or consistent use of ICS 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 subjects who did consistently use ICS. Among children with a normal enrollment FENO (< 25 ppb), there was no difference in final lung function between children who used ICS consistently and those who did not. At 2 and 4 weeks following hospital discharge FENO was higher in subjects who reported albuterol use more than twice weekly. Finally, FENO was higher at 2 and 4 week follow-up visits among subjects who reported that they were not taking ICS.

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

There are few studies that have assessed EBC CysLT concentrations during acute asthma exacerbations¹¹ or following 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 average 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 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 to 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 subjects, and EBC CysLT concentrations are often below the detection limit of enzyme immunoassays (EIA) or liquid chromatography (LC) ²⁶ in many asthmatic and/or healthy subjects.^{9,10,27,28} This may be due in part 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 as compared to healthy subjects is consistent with past observations. However, with the exception of 8 asthmatic subjects in whom EBC CysLT concentrations decreased following hospitalization, for most subjects CysLT concentrations changed little during the follow-up period. Brussino et al. reported that EBC CysLT concentrations increased following allergen challenge in adult asthmatics. ¹⁵ In most of our subjects viral respiratory tract infection was the presumed trigger of their acute exacerbation. Our subjects 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 measuring 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 that had elevated enrollment FENO (≥ 25 ppb), the consistent use of ICS 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) there was no difference in final lung function independent of ICS use during the follow-up period. These results are consistent with observations in stable adults and children with asthma showing that elevated FENO is predictive of a positive response to treatment with ICS. ^{29–31} Of note, during the follow-up period FENO was significantly higher among subjects that did not consistently use ICS, suggesting that FENO may also have utility as a marker of adherence with prescribed ICS. Given that adherence with ICS among asthmatics is frequently suboptimal, an objective biomarker such as FENO that can predict steroid responsiveness and identify subjects who are not consistently taking ICS 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, subjects did not complete enrollment lung function, measurement of FENO, and collection of EBC immediately upon 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 steroids, we suspect that enrollment FENO concentrations in our patients were lower than what their FENO 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 subjects were recruited during hospitalization for an acute asthma exacerbation, we did not have baseline lung function measurements prior to hospitalization, so we were unable to determine whether subjects truly returned to their baseline lung function. In addition, since we did not perform post-bronchodilator spirometry or methacholine challenge in our subjects we were unable to assess associations between FENO concentrations and bronchodilator responsiveness or airway hyper-reactivity. Although most of our children with asthma reported URI 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 regards to gender or ethnicity, variables which 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 following hospitalization, and concentrations of CysLT in EBC do not correlate with lung function during or following hospitalization. However, in patients with elevated FENO during hospitalization for acute asthma, those who reported consistent use of ICS post-hospitalization had significantly better lung function and used less albuterol one month following hospital discharge than those who did not use ICS. 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.

Acknowledgments

Supported by: This research was conducted with support from the Investigator-Sponsored Study Program of AstraZeneca/AstraZeneca Investigator Initiated Study Grant; NHLBI: K23HL077626; CTSA Grant Number I UL1 RR025014-01

References

1. Bloom B, Cohen RA, Freeman G. Summary health statistics for U.S. children: National Health Interview Survey, 2007. *Vital Health Stat.* 2009; 10(239):1–80.
2. NIH/NHLBI. [Last accessed January 11, 2012] 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>
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(5):963–972. [PubMed: 20394979]
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(4):2793–2800. [PubMed: 19620293]
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(4):399–409. [PubMed: 20336062]
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(1):19–24. [PubMed: 18990678]
7. Kerckmar C. Exhaled nitric oxide in the diagnosis and management of childhood asthma. *Ther Adv Respir Dis.* 2010; 4(2):71–82. [PubMed: 20215487]
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(10):1345–1349. [PubMed: 12406853]
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(2):257–263. [PubMed: 14767439]
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(4):764–770. [PubMed: 15805996]
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(6):505–509. [PubMed: 12775861]
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(1):59–99. [PubMed: 19535666]
13. American Thoracic S, European Respiratory S. 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(8):912–930. [PubMed: 15817806]
 14. Gardner RM, Hankinson JL. Standardization of spirometry--1987 ATS update (American Thoracic Society). *J Occup Med*. 1988; 30(3):272–273. [PubMed: 3361365]
 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(11):1642–1647. [PubMed: 20726860]
 16. Baptist AP, Khan FI, Wang Y, Ager J. Exhaled nitric oxide measurements in hospitalized children with asthma. *J Asthma*. 2008; 45(8):670–674. [PubMed: 18951258]
 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(5):415–419. [PubMed: 18517071]
 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(2):171–176. [PubMed: 18430315]
 19. Strunk RC, Szeffler SJ, Phillips BR, et al. Childhood Asthma R, Education Network of the National Heart L, Blood I. Relationship of exhaled nitric oxide to clinical and inflammatory markers of persistent asthma in children. *J Allergy Clin Immunol*. 2003; 112(5):883–892. [PubMed: 14610474]
 20. Mappa L, Cardinale F, Camodeca R, et al. Exhaled nitric oxide and air trapping correlation in asthmatic children. *Allergy*. 2005; 60(11):1436–1439. [PubMed: 16197478]
 21. Spergel JM, Fogg MI, Bokszczanin-Knosala A. Correlation of exhaled nitric oxide, spirometry and asthma symptoms. *J Asthma*. 2005; 42(10):879–883. [PubMed: 16393728]
 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(4):645–650. [PubMed: 11031334]
 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(5):1216–1217. [PubMed: 17655919]
 24. Cap P, Pehal F, Chladek J, Maly M. Analysis of exhaled leukotrienes in nonasthmatic adult patients with seasonal allergic rhinitis. *Allergy*. 2005; 60(2):171–176. [PubMed: 15647037]
 25. Bucchioni 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(7):651–655. [PubMed: 15250231]
 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(1):134–143. [PubMed: 15958190]
 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(4):1958–1963. [PubMed: 16236841]
 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(4):1334–1340. [PubMed: 14555563]
 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(4):453–459. [PubMed: 15901605]
 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 longterm response to inhaled corticosteroid and leukotriene modifier therapies in pediatric asthma. *J Allergy Clin Immunol*. 2009; 123(2):411–416. [PubMed: 19121860]
 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(1):45–52. [PubMed: 16387583]

FIGURE 1.

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

FIGURE 2.

Lung function trend (panel A = FEV₁ % predicted, panel B = FEF₂₅₋₇₅ % predicted) during and following hospitalization for an acute asthma exacerbation.

Definition of Abbreviations: FEV₁ = forced expiratory volume in 1 second, FEF₂₅₋₇₅ = forced expiratory flow between 25 and 75%

Title

FIGURE 3.

Lung function one month following hospitalization for an acute exacerbation of asthma.

Definition of Abbreviations: FEV₁ = 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 < 25 parts per billion (ppb), high FENO = concentration of exhaled nitric oxide ≥ 25ppb.

* ANOVA p=0.02; Tukey's test p<0.05 for comparison of high FENO (+) ICS group vs. low FENO (-) ICS group.

FIGURE 4.

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

FIGURE 5.

Exhaled nitric oxide (FENO) concentrations at 2 weeks (panel A) and 4 weeks (panel B) following hospitalization for an acute exacerbation of asthma.

Definition of Abbreviations: (-) ICS = patients who did not use inhaled corticosteroids during follow-up, (+) ICS = patients who consistently used inhaled corticosteroids during follow-up.

TABLE I

Subject Characteristics

	Children with asthma completing study	Children with asthma not completing study	Healthy Controls
Subjects (n)	34	6	40
Gender (male)	24 (71%)	3 (50%)	18 (45%)
Age at enrollment (yrs) *	9.7 ± 2.9	10.3 ± 6.3	10.8 ± 3.2
Race/Ethnicity			
Caucasian	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 (0%)	2 (33%)	0 (%)
Atopic by skin prick or RAST testing	22 (65%)	6 (100%)	0 (0%)
Eczema (physician-diagnosed and steroid treated)	14 (41%)	4 (67%)	0 (0%)
Family history asthma (1st degree relative)	23 (68%)	5 (83%)	5 (13%)
Environmental tobacco smoke exposure	9 (26%)	3 (50%)	6 (15%)
History of hospitalization for asthma over the past 12 months	14 (41%)	1 (17%)	N/A
History of intensive care unit stay for asthma	9 (26%)	2 (33%)	N/A
Viral URI symptoms during week prior week prior to admission	27 (79%)	5 (83%)	N/A
Inhaled corticosteroid use during follow-up (budesonide equivalent dose)	18 (53%)	N/A	N/A
200 mcg twice daily	9 (26%)		
400 mcg twice daily	7 (21%)		
1 mg once daily			
2mg once daily	1 (3%)		
2mg once daily	1 (3%)		
Systemic steroid treatment (prednisone 2mg/kg/day or dexamethasone 0.6mg/kg/day) between hospital admission and enrollment study visit	34 (100%)	6 (100%)	N/A
Enrollment FE _{NO} (ppb) * [†]	31.5 ppb ± 26.7	50.8 ppb ± 45.3	7.0 ppb ± 2.4
Enrollment EBC CysLT (pg/mL) * ^{††}	7.9 ± 7.3	8.5 ± 10.2	4.9 ± 3.6
Enrollment FVC (% predicted) * [†]	88.1 ± 18.7	83.3 ± 19.3	101.6 ± 10.0
Enrollment FEV ₁ (% predicted) * [†]	76.9 ± 20.5	72.8 ± 16.3	99.3 ± 10.8
Enrollment FEV ₁ /FVC ratio * [†]	76.2 ± 8	75.8 ± 9.6	90.5 ± 5.3
Enrollment FEF ₂₅₋₇₅ (% predicted) * [†]	43.3 ± 26.7	45.5 ± 25.5	90.7 ± 19.7

Definition of Abbreviations: FE_{NO} = fraction of exhaled nitric oxide; ED = emergency department.

* mean ± standard deviation

[†] p<0.001 for children with asthma vs. healthy controls.

^{††} p=0.03 for children with asthma vs. healthy controls.

Table II

Clinical Outcomes 1 Month Post Hospitalization Based on Enrollment FENO and ICS use During Follow-up

	FENO <25ppb + consistent ICS use during follow-up	FENO <25ppb+ inconsistent ICS use during follow-up	FENO 25ppb + consistent ICS use during follow-up	FENO 25ppb + inconsistent ICS use during follow-up
Subjects (n)	10	8	8	8
Daily Albuterol use	0 (0%)	1 (12.5%)	0 (0%)	4 (50%) *
Missed School	2 (20%)	0 (0%)	1 (12.5%)	3 (37.5%)
FEV1 (% predicted)	98.0 ± 10.5	91.1 ± 12.8	105.0 ± 5.6	85.3 ± 4.7 **
FEF25-75 (% predicted)	74.4 ± 16.1	82.9 ± 23.6	88.1 ± 6.2	61.6 ± 7.5 †
Duration of systemic corticosteroid use prior to enrollment FENO (mean ± s.d.)	33.2 ± 8.6	35.8 ± 10.6	37.1 ± 12.6	35.9 ± 7.2

* P=0.03 compared to Enrollment FENO 25ppb + consistent ICS use group

** ANOVA p=0.02; Tukey's test p<0.05 for comparison of FENO 25ppb + consistent ICS use group vs. FENO 25ppb + inconsistent ICS use group

† ANOVA p=0.02; Tukey's test p<0.05 for comparison of FENO 25ppb + consistent ICS use group vs. FENO 25ppb + inconsistent ICS use group.