Original Article

The Effect of Viral Infection on Exhaled Nitric Oxide in Children with Acute Asthma Exacerbations

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What is already known about this topic? Fraction of exhaled nitric oxide (FENO) is a tool that can be used to diagnose asthma and to predict a positive response to inhaled steroid therapy, and can help measure adherence with inhaled steroid therapy in patients with chronic asthma. Unfortunately, its utility in the management of acute asthma has not been widely studied.

What does this article add to our knowledge? This study found that in children with an acute asthma exacerbation, FENO levels are elevated compared with baseline levels. In addition, FENO levels rise to a greater extent in children who do not have a viral-induced asthma exacerbation, suggesting that eosinophilic inflammation plays more of a role in non−viral-induced asthma exacerbations. Last, prednisone was effective in reducing FENO levels to their baseline state, with the biggest reduction seen in those with viral infections.

How does this study impact current management guidelines? This study suggests that FENO is a useful marker of inflammation during an acute asthma exacerbation and that prednisone therapy improves lung function and normalizes FENO levels in children with acute asthma regardless of the underlying cause of their exacerbation.

BACKGROUND: Fraction of exhaled nitric oxide (FENO) level is used as an aid in the diagnosis and management of chronic asthma. Its role in acute asthma remains to be studied.
OBJECTIVE: To determine whether FENO levels are elevated in children with asthma exacerbations compared with baseline, and whether there is a difference in FENO levels based on PCR positive ($+$) (respiratory virus isolated by PCR analysis) versus PCR negative ($-$) (respiratory virus not isolated by PCR analysis) status.
METHODS: Children with a previous FENO level measurement while stable and who presented to an urgent care facility with an asthma exacerbation were enrolled. FENO levels, spirometry, and nasal swabs for viral PCR were obtained at the time of the exacerbation and following a course of prednisone. Data were available on 66 children. Linear mixed models were used to regress the outcomes of interest (FEV_1, FEV_1/forced vital capacity, forced expiratory flow at 25% to 75% of forced vital capacity, and natural log FENO) on detected virus (yes/no), visit (baseline, exacerbation, follow-up), and the interaction between the detected virus and visit.
RESULTS: Compared with baseline, higher FENO values and lower lung function were found at the time of an exacerbation. A respiratory virus was detected in 59% of the exacerbations. The interaction between PCR ($+$) and PCR ($-$) groups and visit on log FENO was marginally significant ($P = .07$). There was no difference in log FENO between the PCR ($+$) and PCR ($-$) groups at baseline, while higher log FENO was found in the PCR ($-$) group at the time of exacerbation and following prednisone ($P = .05$ and .001, respectively).
CONCLUSIONS: Higher FENO concentration in PCR ($-$) exacerbations suggests an eosinophilic predominance in nonviral compared with viral exacerbations.   © 2015 American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2015;3:913-9)

Abbreviations used
ED- emergency department
FEF_{25-75}- forced expiratory flow at 25% to 75% of forced vital capacity
FENO- fraction of exhaled nitric oxide
FVC- forced vital capacity
GC- glucocorticoid
PCR (+)- respiratory virus isolated by PCR analysis
PCR (−)- respiratory virus not isolated by PCR analysis

Asthma is a chronic inflammatory disorder most commonly associated with eosinophilic infiltration into the airways and the release of several inflammatory mediators,[1] although other phenotypes of asthma are described, that is, neutrophilic and paucigraniolocytic. Fractional exhaled nitric oxide (FENO) has been accepted as a noninvasive measure of airway inflammation that can be used in both the diagnosis and the management of asthma.[2,3] Studies have shown FENO to be as effective as beta-agonist reversibility and methacholine responsiveness in diagnosing asthma in both children and adults.[4-6] FENO levels are positively correlated with bronchial wall inflammation,[7] induced-sputum eosinophilia,[8-10] and airway hyperresponsiveness.[11-13] Increases in FENO are associated with deterioration in asthma control with poor inhaled glucocorticoid (GC) adherence,[2,11] whereas FENO levels fall in a dose-dependent manner with inhaled GC treatment.[11,12] Despite enhanced knowledge of FENO in chronic asthma, little is known regarding its role in acute asthma. In 1 of 2 recently published studies, FENO was not found to be useful in acute asthma because it could be measured only in 68% of the children presenting to the emergency department (ED) with acute asthma,[14] while in the second study, FENO failed to distinguish children requiring hospitalization from those who were discharged to home.[15] In neither study were FENO levels available at baseline, nor an attempt was made to determine what role viral infections played in FENO levels. The aims of this study were 2-fold: to determine whether the FENO level is elevated during an acute asthma exacerbation compared with baseline in children with persistent asthma and to determine the influence of viral infections on FENO levels in children presenting with an acute asthma exacerbation requiring prednisone therapy.

METHODS
Study design

In this prospective cross-sectional study, informed consent was obtained from the parent or guardian and assent was obtained from the child when appropriate. The study was reviewed and approved by the National Jewish Health Institutional Review Board. The study was performed at the National Jewish Health Urgent Care Clinic from June 2010 to May 2011. A total of 70 participants aged 7 to 18 years were enrolled. We chose this age group because children of this age can perform adequate spirometry and exhaled nitric oxide measurements.

All patients who presented to the Urgent Care Clinic at National Jewish Health for an acute asthma exacerbation and who had undergone spirometry and FENO measurements within the last 6 months when clinically stable (visit 1) were approached to participate in the study (Figure 1). Patients who could not perform either spirometry or FENO measurements at the time of the exacerbation were ineligible as were patients who were on or had been treated with prednisone within 1 week before the urgent care visit. Children with a history of lung disease other than asthma and patients with a

history of vocal cord dysfunction were also excluded from participating in the study. Once consent and assent were obtained, participants completed a clinical questionnaire, spirometry, and FENO measurement and had a nasal swab obtained for viral PCR (visit 2). Urgent care physicians independently directed treatment and determined the participants' dispositions. The treating physicians were not study investigators and the decision to treat with prednisone was at the discretion of the treating physician using treatment algorithms based on the National Asthma Education and Prevention Program guidelines.[16] Participants who were discharged home were instructed to continue usual controller medications, to take albuterol at a frequency directed by the urgent care physician, and to complete a 5- to 7-day course of oral prednisone (2 mg/kg/d; maximum daily dose 60 mg). All discharged participants were also scheduled for follow-up 1 week later (visit 3). At the follow-up visit, all participants underwent spirometry and had FENO levels measured.

Lung function measurement

All participants underwent spirometry with at least 3 acceptable maneuvers. The three highest forced vital capacity (FVC) and FEV_1 values were recorded according to the American Thoracic Society Guidelines[17] using a Jaeger MasterScreen Pneumo running JLab 5.20 software (Erich Jaeger, Inc, Wurzburg, Germany). The data for FEV_1, FVC, and forced expiratory flow at 25% to 75% of forced vital capacity (FEF_{25-75}) were expressed as % predicted using the NHANES III reference values.[18]

FENO level measurement

The FENO level was obtained after spirometry, and before the administration of short-acting beta-agonist therapy. The FENO level was measured using the online technique recommended by the American Thoracic Society with the Niox Mino system (Aerocrine AB, Stockholm, Sweden).[19] This technique uses a resistive device that provides a constant low expiratory flow rate and vellum closure. The combination of vellum closure and low flow rate ensures accurate measurement of pulmonary-derived exhaled NO levels and excludes contamination from nasal NO. Participants exhale to their residual volume, insert the mouthpiece, inhale to total lung capacity, and then exhale for 10 seconds at a steady rate of 50 mL/s. Visual incentives provide feedback for flow rate compliance. The end point of measurement occurred when a plateau for 4 seconds was observed. Only 1 measurement was obtained because the repeatability of FENO obtained with the Niox Mino is very high.[20]

Viral PCR measurement

Nasopharyngeal swabs were collected using Copan flocked swabs (Copan Diagnostics, Inc, Murrieta, Calif) placed in Universal Transport Medium. These samples were extracted using the Quigen MinElute Virus Kit (Quiagen, Inc, Valencia, Calif) and tested for the presence of respiratory viral nucleic acid using the Quigen ResPlex II v2.0 Kit. This kit can detect the following respiratory viruses: respiratory syncytial virus, influenza A virus, influenza B virus, rhinovirus/enterovirus, parainfluenza virus, human metapneumoviruses A and B, coxackievirus/echovirus, adenovirus, coronavirus, and bocavirus.

Statistical analysis

Comparisons between PCR (+) (respiratory virus isolated by PCR analysis) and PCR (−) (respiratory virus not isolated by PCR analysis) groups at baseline and at the time of an exacerbation were evaluated using independent sample t tests (for normally distributed continuous variables), or Wilcoxon rank-sum tests (for variables that were not normally distributed), and using either χ^2 tests or the
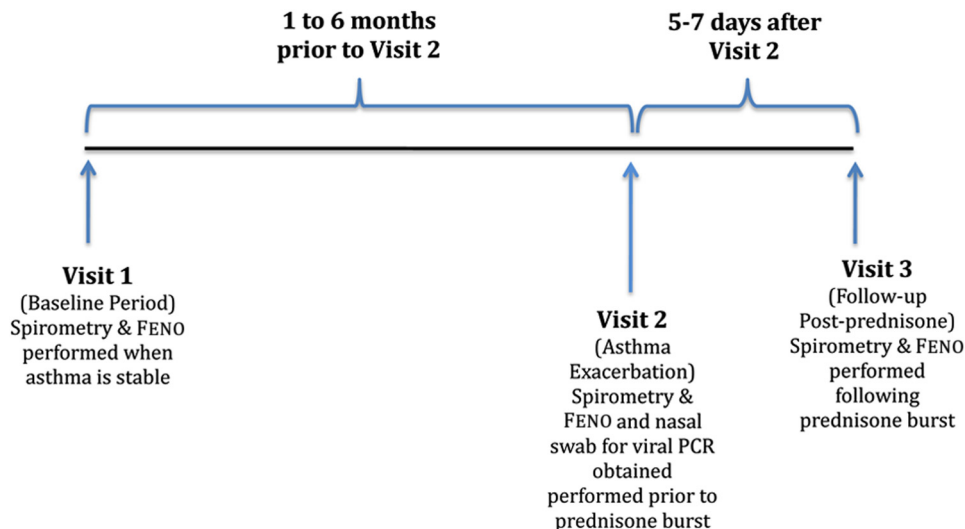
FIGURE 1. Schematic of study design.

TABLE I. Patients' demographic characteristics at visit 1

Characteristic	Entire cohort (n = 66)	PCR (−) (n = 27)	PCR (+) (n = 39)	P value
Age (y)	11 ± 3.2	10.8 ± 2.4	11.1 ± 3.7	.80
Sex: male, n (%)	44 (67)	18 (67)	26 (67)	1.0
Race/ethnicity (white/Hispanic/black)	43/38/18	46/31/23	41/44/15	.53
BMI (kg/m^2)	20.0 ± 6.3	19.2 ± 3.9	20.5 ± 7.6	.36
Inhaled GC dose (µg)	360 [200, 640]	410 [200, 670]	360 [200, 640]	.80
Atopic, n (%)	50 (88)	19 (86)	31 (89)	.80
Number of positive skin test results	9.5 ± 7.3	9.1 ± 8.3	9.8 ± 7.1	.89
Need for prednisone past 6 mo, n (%)	25 (39)	12 (46)	13 (34)	.34
ED visits past year, n (%)	25 (40)	10 (38)	15 (41)	.87
Hospitalizations ever, n (%)	29 (46)	9 (35)	20 (54)	.13
FVC (% predicted)	103.0 ± 14.0	102.9 ± 12.8	103.1 ± 14.8	.95
FEV$_1$ (% predicted)	95.0 ± 16.0	95.7 ± 16.5	94.5 ± 15.9	.76
FEV$_1$/FVC (%)	80.2 ± 9.9	80.9 ± 10.2	79.8 ± 9.9	.68
FEF$_{25-75}$ (% predicted)	76.6 ± 26.8	78.7 ± 27.3	75.2 ± 26.7	.61
F$_{ENO}$ (ppb)	18.5 (14.0, 32.0)	20.0 (14.0, 31.0)	18.0 (10.0, 34.0)	.62

BMI, Body mass index; *ppb*, parts per billion.
Mean ± SD reported unless stated otherwise. For F$_{ENO}$ and inhaled GC dose, median (first quartile, third quartile) reported. *P* value from χ^2 test for categorical variables and 2-sample *t* test with unequal variance for continuous variables. For F$_{ENO}$ and inhaled GC dose, *P* value from Wilcoxon 2-sample test.

Fisher exact test (for small expected numbers) for categorical variables.

Linear mixed models were used to regress the outcomes of interest (FEV$_1$, FEV$_1$/FVC, FEF$_{25/75}$, and natural log F$_{ENO}$) on detected virus (yes/no), visit (baseline, exacerbation, follow-up), and the interaction between the detected virus and visit. The interaction was the main predictor of interest in the models, and was kept in the model even if it was not statistically significant to allow for model flexibility. Covariates of interest for adjusted models were season (winter, spring, summer, and fall) and inhaled GC therapy (yes/no), and also sex, weight, height, and race (Hispanic, African American, or Caucasian) for the adjusted F$_{ENO}$ model. Backwards selection using a *P*-value cutoff of .15 was used to remove insignificant covariates. Final adjusted models included season as a covariate. In addition, the adjusted F$_{ENO}$ model contained age and race

(Hispanic, African American, or Caucasian) as covariates. All models contained a random intercept. To account for repeated measures and unequal times between visits across subjects, a Spatial Power covariance structure based on days from exacerbation was used. All analyses were done in SAS version 9.4. Plots were generated in R version 3.1.0.

RESULTS

Between June 2010 and May 2011, 70 participants were enrolled (Table I). Five subjects did not return for visit 3 and were not included in the analysis. The mean age was 11 ± 3.2 years, 67% were male, 88% were atopic (presence of ≥1 positive skin test result to an aeroallergen), and 31% were obese. Forty-three percent of the enrolled children were Caucasian, 38%

TABLE II. Symptoms, lung function, and F_{ENO} level at visit 2 (time of asthma exacerbation)

Parameter	All	PCR (−)	PCR (+)	P value
Daytime symptoms/wk	3 (2, 5)	4 (2, 7)	3 (1.8, 4)	.11*
Nighttime symptoms/mo	3 (2, 6)	4 (2, 7)	2 (1.8, 3.2)	.02*
Duration of symptoms requiring rescue albuterol	1 (1, 3)	2 (1, 3)	1 (1, 2)	.23*
FVC (% predicted)	91 ± 2	95 ± 3	87 ± 3	.10
FEV_1 (% predicted)	72 ± 2	74 ± 3	69 ± 3	.19
FEV_1/FVC (%)	68 ± 1	67 ± 2	68 ± 2	.80
FEF_{25-75}	43 ± 2	43 ± 3	42 ± 3	.83
Change in FEV_1 following albuterol	29 ± 2	36 ± 3	24 ± 3	.002
F_{ENO} (ppb), median (25th, 75th %tile)	59 ± 5 43 (21, 77)	70 ± 10 68 (35, 89)	49 ± 9 39 (15, 67)	<.001

Values presented as medians (first, third quartile) or means ± SEM. Comparison between PCR (−) and PCR (+) groups using independent-samples t test or χ^2 test.
*Comparison between PCR (−) and PCR (+) groups using the Wilcoxon test.

TABLE III. Estimates of F_{ENO} level and lung function at baseline, exacerbation, and after the initiation of prednisone using linear mixed model

Parameter	Baseline*	Exacerbation*	After initiation of prednisone*	P value†	Baseline vs exacerbation‡	Baseline vs after initiation of prednisone‡	Exacerbation vs after initiation of prednisone‡
F_{ENO} (n = 63)				.07			
Overall	22.2 (2.1)	47.5 (7.5)	21.9 (4.3)		25.3 (2.8), P < .0001	1.0 (3.0), P = .99	−25.6 (3.5), P < .0001
PCR (+)	20.2 (2.7)	36.4 (5.3)	14.8 (2.7)				
PCR (−)	21.5 (4.1)	52.1 (8.7)	29.0 (5.3)				
FEV_1 (N = 66)				.7			
Overall	97.48 (3.63)	73.54 (3.62)	94.23 (3.74)		−23.94 (2.13), P < .0001	−3.25 (2.23), P = .1	+20.69 (2.18), P < .0001
PCR (+)	97.23 (3.93)	71.53 (3.90)	93.07 (3.99)				
PCR (−)	97.72 (4.41)	75.54 (4.38)	95.39 (4.58)				
FEV_1/FVC (N = 66)				.6			
Overall	80.73 (2.16)	68.62 (2.16)	78.03 (2.20)		−12.11 (1.43), P < .0001	−2.69 (1.28), P = .07	+9.42 (1.20), P < .0001
PCR (+)	79.99 (2.35)	68.80 (2.33)	77.06 (2.36)				
PCR (−)	81.47 (2.65)	68.43 (2.63)	79.01 (2.71)				
$FEF_{25/75}$ (N = 66)				.2			
Overall	75.84 (4.78)	41.56 (4.77)	70.99 (4.92)		−34.28 (3.48), P < .0001	−4.85 (3.66), P = .2	+29.42 (3.36), P < .0001
PCR (+)	73.20 (5.28)	41.18 (5.20)	64.76 (5.30)				
PCR (−)	78.48 (5.92)	41.95 (5.88)	77.21 (6.24)				

*Mean (SE) reported. For F_{ENO}, mean (SE) reported. Estimates correspond to the fall season for FEV_1, FEV_1/FVC, and FEF_{25-75}, and average age of 10.9 y, white ethnicity, and the fall season for F_{ENO}.
†P value for interaction of detected virus with visit from linear mixed model. For F_{ENO}, significant differences found between PCR (+) and PCR (−) at exacerbation and after the initiation of prednisone ($P = .05$ and .001, respectively).
‡Mean difference (SE) reported. For F_{ENO}, mean difference (SE) reported. P value comparing visits from linear mixed model, averaged across PCR (+) and PCR (−) subjects.

were Hispanic, and 18% were African American. Fourteen percent carried the diagnosis of mild-persistent, 67% moderate-persistent, and 18% severe-persistent asthma. Seventy-five percent of the subjects were on inhaled GC therapy (15% on inhaled GC monotherapy and 60% on combination inhaled GC/long-acting beta-agonist therapy). More than 40% reported having an ED visit for acute asthma in the past year, requiring systemic steroids in the last 6 months, and having a history of hospital admissions for acute asthma. Review of medication use at baseline (visit 1) compared with the time of exacerbation (visit 2) found no significant difference in the percentage of study participants on inhaled GC therapy alone or in combination with a long-acting beta-agonist, nor were significant differences found in the inhaled GC dose at visit 1 compared with visit 2. In addition, both F_{ENO} level and lung function were in the normal range at visit 1 (geometric mean F_{ENO} level = 20.7 ppb; FEV_1 = 96% predicted; FEV_1/FVC ratio = 81%).

Four of the 70 subjects were missing recorded calendar dates for their visits and were excluded from the mixed models (total of 66 subjects for FEV_1, FEV_1/FVC, and FEF_{25-75} models). In addition, 2 subjects were missing both baseline and follow-up F_{ENO} measurements and 1 subject was missing race information (total of 63 subjects for F_{ENO} models).

At visit 2, the subjects had median daytime symptoms of 3 d/wk, nighttime symptoms 3 times a month, and required rescue albuterol for 1-day duration. Compared with baseline, a significant increase in log exhaled NO (+0.76), and decrease in lung function (FEV_1 % predicted, −24; FEV_1/FVC, −12%;, and FEF_{25-75} % predicted, −34), was noted at the time of exacerbation (all P values <.001) (Tables II and III).

At visit 3, a significant decrease in log exhaled NO (−0.76) and improvement in lung function parameters (FEV_1 % predicted, +21; FEV_1/FVC, +9%; and FEF_{25-75} % predicted, +29, respectively) (P < .001) were found from the
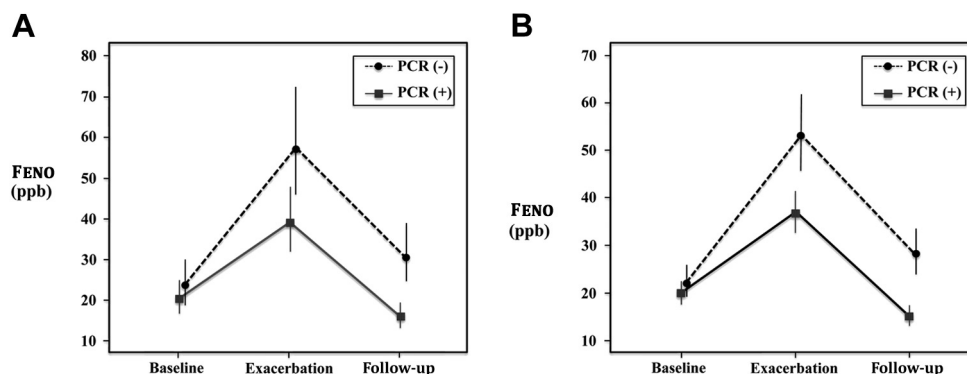
FIGURE 2. A, Exhaled nitric oxide levels at baseline, during an exacerbation, and following a course of prednisone in unadjusted models. Plotted values are mean estimates (± standard errors) from unadjusted linear mixed models. Black circles and dotted lines correspond to the PCR (−) group; gray squares and solid lines correspond to the PCR (+) group. **B**, Change in exhaled nitric oxide levels from baseline, during an exacerbation, and following a course of prednisone in adjusted models. Plotted values are mean estimates (± standard errors) from adjusted linear mixed models. All models have been adjusted for season (fall); FENO model also adjusted for age and race. Plotted mean estimates for FENO correspond to white subjects of mean age 10.9 years. Black circles and dotted lines correspond to the PCR (−) group; gray squares and solid lines correspond to the PCR (+) group. *ppb*, Parts per billion.

time of exacerbation measurement and after the initiation of prednisone (Table III). There were no differences between log FENO (P = .99), FEV$_1$ % predicted (P = .1), FEV$_1$/FVC (P = .07), and FEF$_{25-75}$ % predicted (P = .2) after the initiation of prednisone treatment and baseline measurements.

Fifty-six percent of the children had evidence for a PCR (+) exacerbation, with 1 or more respiratory viruses identified by PCR. No differences in patients' demographic characteristics were noted at baseline between children with PCR (+) versus PCR (−) asthma exacerbations (Table I). The most commonly identified virus was rhinovirus, occurring in 62% of the participants followed by corona virus in 13%, influenza or metapneumovirus in 10%, and parainfluenza in 5%. Of the 39 children with positive viral PCR, 11 had more than 1 virus identified at the time of the exacerbation.

Using unadjusted and adjusted models, changes in the following outcomes (log FENO, FEV$_1$, FEV$_1$/FVC, and FEF$_{25-75}$) were evaluated between PCR (+) and PCR (−) groups. In the unadjusted log FENO model, the interaction between PCR (+) and PCR (−) groups and visit was marginally significant (P = .06; Figure 2, *A*). A trend was also found using adjusted analysis (P = .07; Figure 2, *B*). In both the unadjusted and adjusted models, there was no difference in log FENO between the 2 exacerbation groups by PCR viral detection at baseline (or steady state), but higher log FENO was found in PCR (−) than in PCR (+) children at the time of exacerbation and after the initiation of a prednisone course (P = .05 and .001, respectively, from adjusted analysis). No differences were found between PCR (−) and PCR (+) groups in the measures of lung function studied across visits.

DISCUSSION

In this study of children with asthma, we sought to determine whether FENO levels were elevated during acute asthma exacerbations. We also sought to determine the role PCR (+) exacerbations had on FENO levels and, last, we sought to determine the effect of prednisone on FENO levels and whether there were

differences in response to prednisone based on whether the exacerbation was PCR (+). We found FENO levels to rise significantly during an acute exacerbation of asthma, with the biggest increase noted in PCR (−)-associated exacerbations. Following a short course of prednisone therapy, FENO levels fell to near baseline levels, with the greatest reduction in PCR (+) exacerbations.

Few studies have sought to evaluate FENO levels in acute asthma. Lanz et al[21] found FENO levels to be elevated in children with acute asthma compared with levels in atopic children without asthma and normal control children and following a course of prednisone, FENO levels fell to those of controls without asthma. Limitations of this study included the small number of children studied and the lack of preexacerbation FENO levels. Kwok et al,[14] using a tidal breathing technique to assess FENO levels in children presenting to the ED with an asthma exacerbation, found that only 68% of the children were able to successfully perform the maneuver. Of those who were able to provide an adequate sample, no differences were found in FENO levels among those with mild, moderate, and severe exacerbations. This study was limited by the technique used to measure FENO levels and the lack of baseline FENO levels. Nelson et al[15] sought to determine whether FENO levels at the time of an ED visit could predict children requiring hospitalization for acute asthma and found that FENO levels could not distinguish children requiring hospitalization from those who were discharged to home. This study was limited by the small number of children who required hospitalization compared with those who were discharged to home.

Our study is novel in that we collected baseline, exacerbation, and postprednisone FENO levels in addition to attempting to determine the etiology of the acute asthma exacerbation. We found FENO levels to be the highest in children with acute asthma exacerbations that were not associated with viral infections [PCR(−)]. In this group of children, the exacerbation was likely allergen-driven. In this situation, the offending allergen triggers both immediate and late-phase allergic responses, leading to bronchoconstriction and airway edema as a consequence of

preformed mediators of inflammation and the influx of eosino-phils. Because FENO levels serve as a surrogate for eosinophilic inflammation,[6-9] elevated FENO levels likely represent an influx of eosinophils and eosinophil-derived airway epithelial damage.

FENO levels were also increased (albeit to a lesser extent than PCR(−)-driven exacerbations) in PCR (+) exacerbations of asthma, with this increase coming largely from children infected with rhinovirus. Viral-induced asthma exacerbations are often associated with the influx of neutrophils into the airways.[22,23] In adults with asthma in whom sputum induction was performed at the time of an acute exacerbation, neutrophilic inflammation was noted in 68% of the cases.[24] This may not come as much of a surprise because up to 80% of acute asthma exacerbations are caused by respiratory viruses.[25-27] Past studies in adults with asthma have demonstrated that neutrophil-driven inflammation is not associated with elevated FENO levels.[28]

The mechanisms by which viral infections contribute to changes in airway inflammation and altered physiology that result in asthma exacerbations remain incompletely under-stood.[29] Message et al[30] addressed this issue by inoculating those with asthma and healthy controls with human rhinovirus. Both groups developed respiratory symptoms, but those with asthma had lung function impairment and heightened bronchial responsiveness and developed eosinophilic inflammation. Unlike respiratory syncytial virus and influenza virus, rhinovirus is unique in that infection in those with asthma results in increased FENO levels[31-33] by way of increased inducible nitric oxide syn-thase expression in airway epithelial cells.[34] Indeed, when chil-dren with viral-associated exacerbations caused by rhinovirus were excluded from those with other respiratory viruses, FENO levels were not elevated.

Both viral infections and allergic inflammation can damage the airway epithelium, which results in asthma worsening. Viral-induced damage to the airway epithelium can lead to enhanced absorption of allergens, resulting in worsening airway inflam-mation. Likewise, damage of the airway epithelium from allergic inflammation may promote viral replication and more severe clinical illness.[29] Thus, in our children with PCR (+) asthma exacerbations, there is likely to be a mixture of both eosinophilic and neutrophilic inflammation, which results in the moderate elevation in FENO levels we noted. In contrast, the inflammation underlying PCR (−) exacerbations is more likely to be a purely eosinophil-driven process and as a result, FENO levels would be expected to be higher.

Our study has a number of limitations. First, from a meth-odological standpoint, it would have been preferable to perform FENO measurements before spirometry because spirometry can result in a transient reduction in FENO levels. Past studies have shown the reduction to be modest (2 ppb or <10%)[35] and unlikely to have significantly altered our results. Second, we did not inquire as to whether our patients had recently ingested foods containing high levels of nitrates, or whether they were exposed to household cigarette smoke or were currently smoking ciga-rettes. Ingestion of foods containing high levels of nitrates can cause the FENO level to be elevated for up to 15 hours,[36] while current cigarette smoking can reduce the FENO levels by 60%.[37] In addition, we reported the FEV_1/FVC ratio as a percentage, as opposed to the FEV_1/FVC percent predicted. Because the ratio varies with age and sex, using percentage only misses this in-formation and can give a false sense of abnormalities. Another limitation is that we were unable to identify the triggering

agent(s) involved in the acute exacerbation in the PCR (−) group. It is possible that these subjects were reacting to inhaled seasonal allergens or newly acquired perennial allergens such as cat or dog dander. Alternatively, some of our patients may have had a falsely negative PCR result. Last, although we speculate that PCR (−) exacerbations were likely eosinophil driven, we did not perform sputum induction or collect peripheral blood to assess for eosinophilia.

In conclusion, FENO levels are elevated in acute childhood asthma, with higher levels seen in children with PCR (−) ex-acerbations. A short course of prednisone resulted in significant reductions in FENO levels. Future studies are needed to better understand the underlying pathophysiologic differences between PCR (+) and PCR (−) asthma exacerbations. This is of potential importance because therapy may be altered on the basis of the underlying inflammatory process. By providing optimal therapy at the time of an exacerbation, attenuation of airway remodeling and loss of lung function as a result of the exacerbation may be achieved.

Acknowledgments

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