Assessment of fractionated exhaled nitric oxide as a biomarker for the treatment of eosinophilic esophagitis

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ABSTRACT

Diagnosis of eosinophilic esophagitis (EoE) and determination of response to therapy is based on histological assessment of the esophagus, which requires upper endoscopy. In children, in whom a dietary approach is commonly used, multiple endoscopies are needed, because foods are eliminated and then gradually reintroduced. Ideally, noninvasive methods could supplement or replace upper endoscopy to facilitate management. Fractionated exhaled nitric oxide (FeNO) has been proposed as a useful measure for monitoring disease activity in studies of patients with eosinophil-predominant asthma and in other atopic disorders. Thus, we evaluated whether FeNO levels could be a useful biomarker to assess the response to therapy in EoE patients. This study was designed to determine whether there is a change in FeNO levels during treatment with topical corticosteroids and whether changes correlated with clinical response. This was a prospective, multicenter study that enrolled nonasthmatic patients with established EoE. FeNO levels and symptom scores were measured at baseline, biweekly during 6-week swallowed fluticasone treatment, and 4 weeks posttreatment. Twelve patients completed the trial. We found a statistically significant difference between median pre- and posttreatment FeNO levels [20.3 ppb (16.0–29.0 ppb) vs 17.6 ppb (11.7–27.3 ppb), p=0.009]. However, neither the pretreatment FeNO level, a change of FeNO level after 2 weeks of treatment, nor the FeNO level at the end of treatment confidently predicted a clinical or histological response. Although our findings suggest nitric oxide possibly has a physiological role in EoE, our observations do not support a role of FeNo determination for management of EoE.

(Allergy Asthma Proc 33:519–524, 2012; doi: 10.2500/aap.2012.33.3606)

Eosinophilic esophagitis (EoE) is a chronic, immune/antigen-mediated esophageal disease characterized clinically by symptoms related to esophageal dysfunction and histologically by eosinophil-predominant inflammation.[1] The primary treatment options are topical corticosteroids and elimination of implicated foods. Diagnosis of EoE and assessment of the response to therapy is based on histological assessment of the esophagus, which requires upper endoscopy. In children, in whom a dietary approach is commonly used, multiple endoscopies are often needed, because foods are eliminated and then gradually reintroduced. Ideally, noninvasive methods could supplement or replace upper endoscopy to facilitate management.

Fractionated exhaled nitric oxide (FeNO) has proven to be a useful measure of disease activity in studies of patients with eosinophil-predominant asthma.[2] FeNO levels are also increased in other atopic diseases in which eosinophils are thought to have an important role.[3,4] Thus, we sought to evaluate whether FeNO levels could be a useful biomarker to assess the response to therapy in patients with EoE.

PATIENTS AND METHODS

Study Design and Study Participants

This was a prospective, multicenter pilot study that enrolled nonasthmatic patients with established EoE. Patients aged 7–65 years were recruited from the Tufts Medical Center, Floating Hospital for Children, Boston, MA, and the Brigham and Women's Hospital, Boston, MA. All patients and all parents of adolescent patients provided written informed consent. The study

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Funded by Tufts CTSI's Pilot Studies Program (i.e., Catalyst, Planning, or Methods Development); the project was funded by the National Center for Research Resources and the National Center for Advancing Translational Sciences, National Institutes of Health, through Grant Number UL1 RR025752. P.A. Bonis and J. Leung are funded by The International Gastrointestinal Eosinophil Researchers (TIGERS) Concept Award. J. Leung (post-doctoral) was funded in part by National Institutes of Health Ruth L. Kirschstein Institutional Research Training Grant (T32 NIH) T32DK007542. The remaining authors have no conflicts of interest to declare pertaining to this article

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was approved by the Institutional Review Committee at Tufts Medical Center in Boston, MA.

Patients with a clinical history consistent with EoE and esophageal biopsy specimens showing a baseline peak count of esophageal intraepithelial eosinophils (peak eosinophil [PE]) ≥15 in at least 1 high-power field (hpf) evaluated at 400× magnification, despite 6–8 weeks of twice-daily dosing of proton pump inhibitor (PPI) therapy were considered eligible. The definition of EoE was consistent with consensus guidelines.[1]

Patients were excluded if they had used inhaled, nasal, or systemic corticosteroids within the past 3 months; had history of smoking; had undergone dietary modification for EoE in the last 6 months; had history of doctor-diagnosed asthma or rhinitis; had a major systemic illnesses (including cirrhosis, renal insufficiency, heart failure, and respiratory disease); or who were pregnant.

All patients were treated with swallowed fluticasone at 440 μg twice daily for 6 weeks. Techniques of swallowing topical corticosteroids were reviewed at initiation of therapy and reinforced during each visit (at 2-week intervals). Weekly phone calls were made during the 10-week trial to advocate medial compliance. All patients remained on twice-daily PPI therapy throughout the study period.

FeNO levels were measured using Niox Mino (Aerocrine AB, Solna, Sweden) in accordance with American Thoracic Society guidelines.[5] Triplicates at 5-minute intervals were obtained for each measurement pretreatment, biweekly during 6-week treatment period, and 4-week posttreatment. Reference ranges for FeNO levels in healthy controls overlap with those observed in patients with asthma.[6] Nevertheless, in adults with asthma, values <25 ppb (20 ppb in children) are associated with decreased responsiveness to corticosteroids compared with those with values >50 ppb (35 ppb in children).[5] Similarly, a reduction of at least 20% in FeNO from baseline values over 50 ppb (or >10 ppb drop for baseline value <50 ppb) correlates with a significant response to anti-inflammatory therapy.[5] Because cutoff values in eosinophilic gastrointestinal disorders have not been evaluated, we did not prespecify threshold ranges but rather examined the changes in FeNO levels during and after therapy.

Patients were instructed to complete a questionnaire to determine symptom score during each time point. We used a scoring system that has previously been studied in patients with EoE,[7] albeit no symptom scoring questionnaire has been fully validated in patients with EoE. Advantages of this scoring system compared with other symptom scores used in esophageal disease are its explicit inclusion of clinical features known to be associated with EoE including dysphagia, chest pain, heartburn, regurgitation, vomiting, and abdominal pain. Each symptom was scored from 0 to 3 (0, absent; 1, three or less times per week; 2, three to six times per week; and 3, daily), with a total possible score of 18.

Patients who continued to be symptomatic after treatment were offered a repeat upper endoscopy. At least four biopsy specimens were taken from the proximal half and lower half of the esophagus. All biopsy specimens were reviewed by a single board-certified pathologist. Patients were considered to be histological responders if the PE count was ≤7/hpf.[8]

Statistical Methods and Data Analysis

All analyses were made using GraphPad Prism Version 5.04 for Windows (GraphPad Software, La Jolla, CA). FeNO level, PE count and symptom scores were represented as median and interquartile range. Using each patient as his/her own control, we compared the FeNO level, PE count, and symptom score at different time points using Wilcoxon matched-paired signed-rank tests. For the *post hoc* analysis, we compared FeNO level, PE count, and symptom score at different time points between treatment responders and nonresponders using Mann-Whitney test. The association between PE count, FeNO, and symptom scores was analyzed using Spearman rank correlation coefficient. A Spearman rank correlation coefficient of 0.5–1 was considered a strong correlation, 0.3–0.5 a weak correlation, and <0.3 no correlation. A value of $p < 0.05$ was considered significant.

RESULTS

Participant Flow and Demographics

Between September 2010 and September 2011, 29 nonasthmatic patients with a clinical history consistent with EoE and esophageal biopsy specimens showing PE count ≥15/hpf were screened for study participation (Fig 1). Fourteen met the eligibility criteria and were enrolled. The reasons for ineligibility were use of corticosteroids in the past three months ($n = 2$), history of asthma ($n = 6$), esophageal eosinophilic infiltration that resolved with high dose PPI ($n = 4$), and patient refusal ($n = 3$).

Two patients dropped out of the study, both within 3 weeks of enrollment. One dropped out because of job relocation to another state and the other because of work schedule conflict. The 12 remaining patients completed the study. One patient developed esophageal candidiasis during the study period and was excluded from our data analysis.

Ten of the 11 patients were adults. Demographics and baseline characteristics are summarized in Table 1. The majority of the patients were male (8/11), treatment naïve (9/11), had esophageal rings (8/11), and

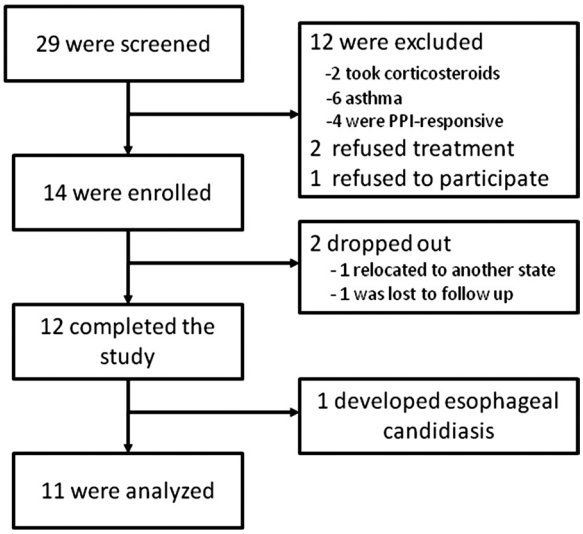

Figure 1. *Participant flow.*

had a history of food impaction (8/11). Overall compliance to medical therapy was reported to be above 95% (a total of 46 missed doses out of a total of 924 expected doses).

Reduction of FeNO Levels and Symptom Score with Treatment

There was a statistically significant difference between the median pre- and posttreatment FeNO level (20.3 ppb [16.0–29.0 ppb] versus 17.6 ppb [11.7–27.3 ppb]; $p = 0.009$; Fig. 2). Two weeks posttreatment, FeNO returned to 23.4 ppb (17.3–34.1 ppb), which was not significantly different from pretreatment level ($p = 0.55$).

Similarly, there was a statistically significant reduction in the median symptom score pre- and posttreatment (3.0 [3.0–6.0] versus 1.0 [0.0–3.0]; $p = 0.006$). Although the median symptom score increased 2 weeks after discontinuation of treatment from 1.0 (0.0–3.0) to 2.5 (0.0–3.5), the change did not reach statistical significance ($p = 0.44$).

Histological Response to Treatment

All 12 patients who completed 6 weeks of treatment reported continued symptoms and elected to undergo a repeat upper endoscopy. One had severe esophageal candidiasis and was excluded from the analysis. Among the remaining 11, only 5 (45%) responded histologically (PE count <7/hpf as defined previously). The pretreatment PE was 23.0 (18.5–36.0) in responders, versus 68 (35.8–102.5) in nonresponders ($p = 0.017$). There was no significant difference between the baseline symptom scores or FeNO levels between responders and nonresponders ($p = 1.0$ and 0.33, respectively; Table 2).

Table 1 **Baseline demographics and clinical characteristics**

Age, yr (SD)	34	(13)
Sex, *n* (%)		
Female	3	(27)
Male	8	(73)
Race, *n* (%)		
White	11	(100)
Black	0	(0)
Asian	0	(0)
Body mass index, mean (SD) (kg/m^2)	26	(4.2)
Smoking, *n* (%)	0	(0)
Duration of EoE, mean (SD) (mo)	12	(20)
Age at onset of EoE, yr	33.2	(12.6)
Treatment naïve, *n* (%)	9	(82)
Concomitant PPI use, *n* (%)	11	(100)
Peripheral eosinophil count, mean (SD) (1000/mm^3)	0.48	(0.27)
Peak esophageal eosinophil count, median (IQR) (cells/hpf)	36	(23–76)
Symptom score, median (IQR)	3.0	(3.0–6.0)
FeNO level, median (IQR) (ppb)	20.3	(16.0–29.0)
History of food impaction, *n* (%)	8	(73)
Frequency of symptoms, *n* (%)		
Dysphagia	11	(100)
Chest pain	2	(18)
Heartburn	7	(64)
Regurgitation	3	(27)
Vomiting	3	(27)
Abdominal pain	4	(36.4)

EoE = eosinophilic esophagitis; PPI = proton pump inhibitor; IQR = interquartile range.

FeNO Levels in Responders and Nonresponders

The median reduction of FeNO levels after treatment was more apparent in histological responders (−26.7%), than in nonresponders (−18.7%), although the difference did not reach statistical significance ($p = 0.08$). We explored whether a reduction of FeNO in the first 2 weeks could predict response to treatment. It was similar in both groups (27.9% in responders versus 26.5% in nonresponders; $p = 0.24$), suggesting initial reduction of FeNO because treatment can not predict treatment response.

There was no significant correlation between FeNO and symptom scores when the data were an-

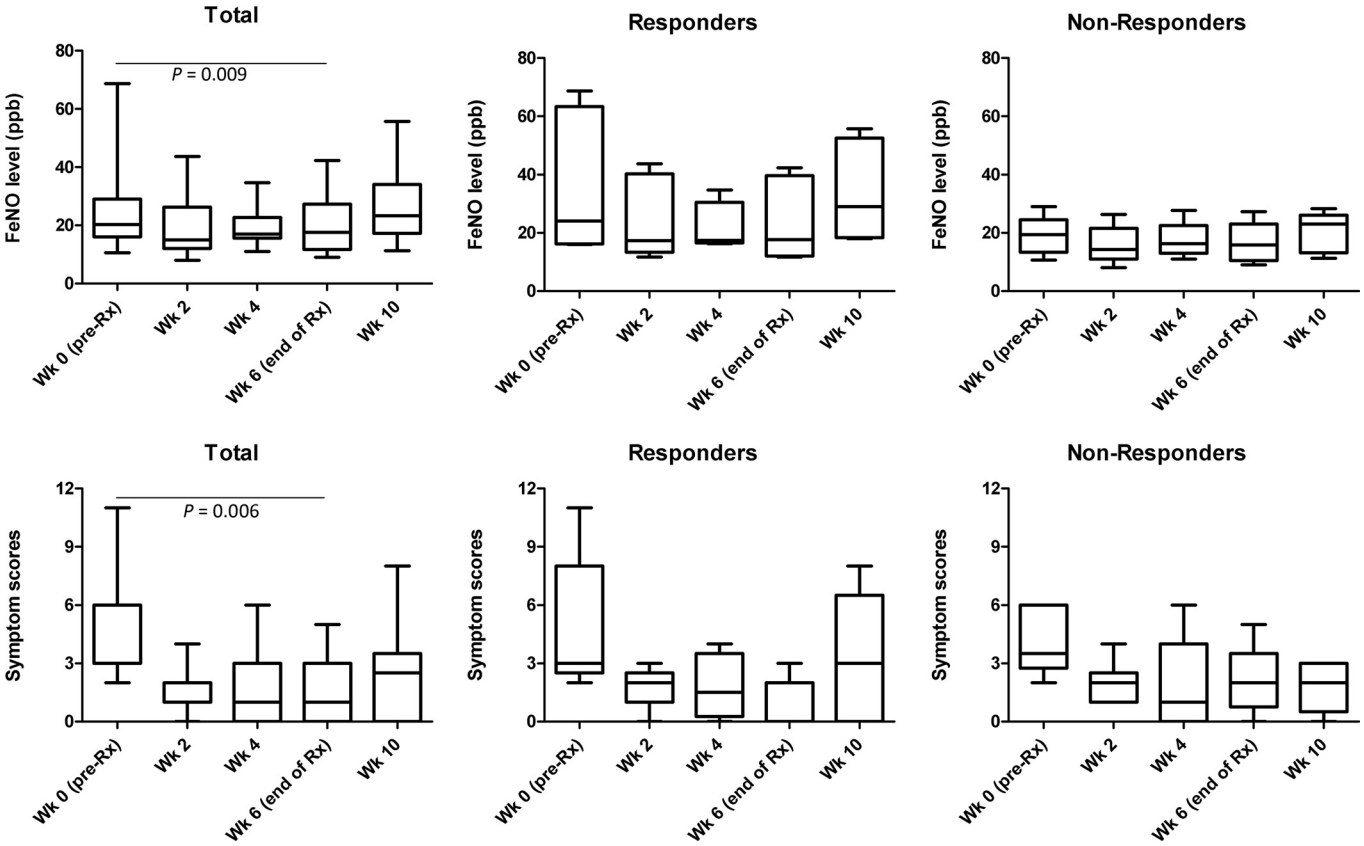

Figure 2. Box and Whisker plot of FeNO levels (upper panels) and symptom scores (lower panels) of all patients, responders and non-responders.

Table 2 **Baseline characteristics of treatment responders and nonresponders**

	Responders	Nonresponders	*p* Value
Peak esophageal eosinophil count, median (IQR) (cells/hpf)	23.0 (18.5–36.0)	68.0 (35.8–102.5)	<0.05
Symptom score, median (IQR)	3.0 (2.5–8.0)	3.5 (2.8–6.0)	1.0
FeNO level, median (IQR) (ppb)			
Week 0	24.0 (16.2–63.4)	19.5 (13.4–24.5)	0.33
Week 2	17.3 (13.4–40.2)	14.3 (11.0–21.6)	0.20

IQR = interquartile range.

alyzed across the entire study group. In a subgroup analysis, we found a modest but significant association between symptom scores and FeNO levels in histological responders ($r = 0.51$; $p = 0.005$; Fig. 3). There was no association between symptom scores and PE count, or FeNO levels with PE count in either group.

DISCUSSION

We found a modest but statistically significant reduction of FeNO level with treatment and a modest correlation between FeNO level and symptom scores among histological responders. In *post hoc* analysis, we found that neither the pretreatment FeNO level nor the change in FeNO level after 2 weeks of treatment predicted treatment response.

In the *post hoc* analysis, we found that the median PE count dropped from 36 (23–76) to 20 (5–60) in all treated patients ($p = 0.029$). This observation suggests that reduction in FeNO levels (20.3 ppb [16.0–29.0 ppb] pretreatment versus 17.6 ppb [11.7–27.3 ppb] posttreatment; $p = 0.009$) might reflect a reduction in eosinophil burden with treatment. Neither systemic[9] nor nasal instilled[10] corticosteroids directly affect FeNO levels in healthy individuals. Thus, the reduction of FeNO level was unlikely to

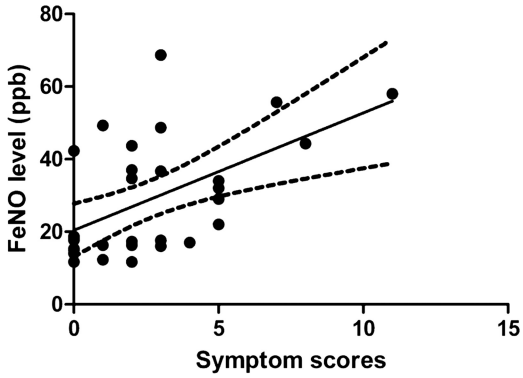

Figure 3. A modest, significant correlation was found between symptom scores and FeNO levels in histological responders.

reflect an effect of corticosteroids on FeNO production. None of the patients enrolled were tobacco smokers or had clinician-diagnosed asthma or rhinitis, which have been associated with elevated FeNO.[5,11] Although we did not include spirometry and/or bronchoprovocation testing as part of the objective evaluation to exclude patients with asthma, the absence of asthma symptoms, history of doctor-diagnosed asthma, or need for inhaled corticosteroids make clinically important asthma unlikely. Nevertheless, it is possible that some of our patients had undetected atopic conditions that may have contributed to the FeNO levels.

Most of the research on FeNO has focused on upper and lower airway diseases. In 1991, Gustafsson and coworkers[12] discovered that NO is present in exhaled breath of humans, and 2 years later Alving and colleagues[13] showed that FeNO is increased in patients with asthma. These findings triggered a great interest in studying various aspects of FeNO and >1000 articles have been subsequently published. In 2005, Smith *et al.*[14] showed in a randomized, single-blinded and placebo-controlled trial that use of FeNO measurements significantly reduced the maintenance doses of inhaled corticosteroids without compromising asthma control, although this finding has recently been challenged[15] and the role of FeNO in guiding asthma management remains to be defined.[16–18]

More recent studies found elevated FeNO levels in patients who were atopic but did not have asthma[6,19]and in nonatopic, nonasthmatic patients with Hodgkin's lymphoma.[20] Atopic dermatitis has also been associated with elevated FeNO levels.[11] These observations suggested a possible role for FeNO in immunologic diseases not restricted to the airways.

The role of NO has been intensively studied in inflammatory bowel disease (IBD). Human and animal studies convincingly showed that NO production is up-regulated in IBD and correlates with disease activity.[21–30] In 2002, Koek *et al.* reported that FeNO levels were elevated in IBD patients and correlated with disease activity,[28] suggesting that gastrointestinal inflammation could elevate FeNO levels. None of our patients had IBD.

In summary, our study found a modest reduction of FeNO level with EoE treatment and a modest significant correlation between FeNO level and symptom scores among histological responders. However, we did not find that pretreatment FeNO level or change in FeNO levels were helpful in predicting treatment response. Thus, while our findings suggest NO possibly has a physiological role in EoE, our observations do not support a role for FeNO determination in the management of EoE.

ACKNOWLEDGMENTS

The authors gratefully thank Robin Ruthazer for assistance with the statistical analysis. Author contributions include the following: J. Leung, study concept and design, acquisition of data, analysis and interpretation of data, drafting of the article, critical revision of the article for important intellectual content, statistical analysis, obtained funding, administrative support, and study supervision; A. Nguyen-Traxler, acquisition of data, critical revision of article, analysis and interpretation of data; E.M. Lee, acquisition of data and critical revision of article; J.S. Yip, acquisition of data and critical revision of article; J.V. Weinstock, study concept and design development, patient recruitment, and critical revision of article; W.W. Chan, patient recruitment and critical review of the article; P. Ngo, patient recruitment, critical review of the article, analysis and interpretation of data; B.J. Weinstein, acquisition of data and critical revision of article; and P.A. Bonis, study concept and design, obtained funding, critical revision of article and study supervision.

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