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ORIGINAL ARTICLE

Poor Relationship Between Fractionated Exhaled Nitric Oxide and Disease Activity in Eosinophilic Esophagitis

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Abstract

Current eosinophilic esophagitis care requires monitoring with repeat endoscopy and biopsy, which has significant cost, risk, and inconvenience for patients. Fractionated exhaled nitric oxide testing (FeNO) is a standardized non-invasive test with proven utility in evaluation of asthma. Elevated FeNO has reported use in other eosinophilic inflammatory conditions; however, its use in eosinophilic esophagitis has not been fully evaluated. To assess the utility of FeNO in predicting severity of eosinophilic esophagitis activity. Fifty patients received fractionated exhaled nitric oxide testing within 1 week of endoscopic evaluation with biopsy for determination of peak eosinophil counts. Presence of furrows was also evaluated with respect to FeNO levels. Spearman correlation was calculated between FeNO and peak eosinophil counts (PEC) with subgroup analysis performed based on PPI use. Spearman correlation was performed on the change in FeNO and PEC on the patients receiving repeat testing. FeNO was poorly correlated to PEC (Spearman correlation 0.22). With a cut-off FeNO value of > 40 ppb, specificity of FeNO for detecting presence of ≥ 15 eos/hpf was 0.94 and sensitivity was 0.16. FeNO showed weak relationship to presence of furrows. Within the subgroup of patients not taking PPI, the spearman correlation was 0.21. Delta- FeNO versus Delta-PEC had spearman correlation of 0.72 for patients receiving repeat testing. FeNO likely has limited clinical utility for predicting severity of esophageal eosinophilia. In patients with FeNO levels > 40 ppb, specificity of testing was high, but very few patients reached this FeNO level.

Keywords Nitric oxide · Eosinophils esophagitis · Eosinophils · Deglutition · Deglutition disorders

Introduction

Eosinophilic esophagitis (EoE) is a chronic inflammatory condition involving an antigen-immune mediated inflammatory process, which leads to esophageal inflammation and remodeling. Both the incidence and prevalence of the disease has increased over the last three decades [1]. EoE can respond to topical steroid and dietary therapy. Currently, endoscopic evaluation with histopathological examination of esophageal biopsy is the gold standard for evaluation of disease activity, required for both establishing diagnosis and for accessing response to treatment [1]. Dietary therapy is effective in 70% of patients with EoE [2, 3]. Allergy testing in adult patients with skin prick test, serum immunocap, or patch testing, is not helpful in identifying food triggers; [4, 5] therefore, multiple endoscopies are required to obtain esophageal histological evidence of response to food restriction and reintroduction. Endoscopy has significant direct and indirect costs as well as inherent risk; making it a less than ideal method for monitoring disease activity. Several less invasive techniques have been trialed including esophageal string testing, the esophageal sponge, and unsedated transnasal endoscopy [6, 7]. Completely non-invasive testing with serum markers of disease have been studied, but are not

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adequately sensitive or specific to predict histologic results [8].

Fractionated oral exhaled nitric oxide (FeNO) is a non-invasive technique, which could be an attractive option for monitoring EoE disease activity due to its ease of administration and standardization. Inducible nitric oxide synthase (iNOS) levels are increased during Th2 mediated responses including allergic and eosinophilic inflammatory processes [8, 9]. The up-regulation of iNOS in the eosinophilic mucosa in patients with EoE leads to increased nitric oxide production, which may be transmitted up the esophagus and mixed with exhaled respiratory gases. This could allow the measurement of FeNO levels to reflect eosinophilic inflammation occurring in the esophagus. Currently, FeNO is used for the diagnosis of eosinophilic airway inflammation, monitoring airway inflammation, and determining likelihood of corticosteroid responsiveness in treatment of chronic respiratory symptoms [10]. Research into the use of FeNO in EoE has included a 55 patient study where 18 of the 55 patients included were found to have a histological diagnosis of EoE; half of which were found to have a FeNO level $\geq$ 15 ppb [11]. However, the study did demonstrate high specificity and negative predictive values, 87 and 78% respectively, for identifying patients without EoE when FeNO level was $<$ 15 ppb [11]. Another small study of 11 patients, found a decrease in FeNO levels and decrease in median peak esophageal eosinophil count in patients with EoE after 6 weeks of treatment with topical steroids [12]. Given the ease of obtaining FeNO level non-invasively; we performed a prospective study to evaluate the role of FeNO in predicting persistent esophageal eosinophilic inflammation in patients with EoE.

Materials and Methods

The study was a prospective single center pilot study approved by the Mayo Foundation Institution Review Board on August 7, 2013. The trial is registered with the ClinicalTrials.gov (NCT01929824). All 50 subjects provided written informed consent. All authors had access to the study data and approved the final manuscript.

Patients

The patients were recruited from the Esophageal Clinic at Mayo Clinic Rochester where they were being evaluated for EoE. Fifty-six patients were recruited between September 2013 and April 2016. All patients had history of symptomatic dysphagia and prior esophageal biopsy with a peak eosinophil count of $\geq$ 15 eos/hpf that persisted despite twice daily PPI therapy. Patients with active asthma were identified during clinical visits prior to receiving FeNO testing, and no patients with active asthma were included in the study. A subset of five patients participated twice at separate time points. All subjects were between 18 and 80 years of age. Fractionated exhaled nitric oxide testing and esophagogastroduodenoscopy (EGD) were performed within 1 week of each other for all patients. Six patients fell outside the 1 week time frame and were excluded. Patients were seen by a core group of 4 gastroenterologists specializing in esophageal disease.

Diagnostic Testing Protocols

All endoscopies were performed with a Mayo staff gastroenterologist in attendance. Biopsies were obtained with standard size biopsy forceps. At least 6 biopsies were obtained from the entire esophagus with at least two samples from the distal esophagus within 5 cm of the visual squamocolumnar junction. All pathology slides were read by one of four GI pathologists experienced in the evaluation of EoE. The biopsy specimens, after processing with hematoxylin and eosin stain, were reviewed using a Nikon E600 microscope with 1025 ultra-wide eyepieces (Nikon, Melville, NY). The area of greatest eosinophil density was identified via low powered review of the slides. Eosinophil counting was conducted using a field area of 0.307 mm^2 and field diameter of 0.625 mm at a 40 objective setting. A peak eosinophil count was reported. FeNO testing was performed according to the American Thoracic Society guidelines using a rapid-response chemiluminescent nitric oxide analyzer (NIOX MINO®).

Analysis

The primary aim of the study was to identify a correlation between FeNO and peak eosinophil counts on biopsy. The secondary aim was to identify the correlation of FeNO to endoscopic measures of EoE activity. Furrows were defined as present or absent. The EOE endoscopic reference score (EREFS) was not incorporated into our clinical practice for most of the study period and therefore, was not reported in the study [13]. Spearman correlation was calculated between the FeNO values and peak eosinophil counts identified by biopsy. Receiver operator curves (ROC) were used to evaluate the relationships between FeNO and the 2 disease makers; peak eosinophil counts $\geq$ 15 eos/hpf and endoscopic findings of furrows. Sensitivities, specificities, positive predictive values, and negative predictive values were also calculated for these relationships. A separate analysis was performed on patients who had undergone repeat FeNO and endoscopy. The change in FeNO values and change in peak eosinophil counts were calculated and a Spearman correlation

coefficient was calculated to assess within-person correlation. Lastly, subgroup analysis using spearman correlation was performed on non-PPI users and PPI users. Analyses were performed using R version 3.3.1 [14].

Results

Demographics of the patients that participated in the trial are provided in Table 1. Sixty-seven percent of the patient came from either Minnesota or Iowa. Despite being histologic non-responders to PPI treatment, several patients were taking PPIs at doses to control GERD symptoms.

The Spearman correlation for peak eosinophil counts vs. FeNO is 0.22 ($P = 0.122$). The scatter plot for FeNO vs peak eosinophil counts is displayed in Fig. 1. The ROC of these variables is displayed in Fig. 2. The area under the curve is 0.65. This ROC was further analyzed at different FeNO cut-off values shown in Table 2. Using a cut-off FeNO > 13.5 ppb to predict a peak eosinophil count ≥ 15 eos/hpf produced a sensitivity of 88% and specificity of 39%. Alternatively, using a cut-off of > 36.5 ppb would improve specificity (89%), but only 25% of patients with peak eosinophils > 15 eos/hpf would have a positive result.

Similarly, presence of furrows was also investigated in relation to FeNO values with creation of a ROC (Fig. 3) and calculation of sensitivity, specificity, positive predictive value, and negative predictive values. These are reported in Table 3. A smaller percentage of furrows was found in the elevated FeNO group (66.7% vs 77%) and sensitivity was low at 11%.

The change in FeNO on repeat testing was calculated and compared to the change in peak eosinophil counts for the patients who had received testing at 2 time points ($n = 5$). A scatter plot was created and spearman correlation was calculated to be 0.62 (Fig. 4). This would be consistent with the hypothesis that within an individual, a relationship exists between EoE activity and FeNO level. However, the trend is heavily influenced by 1 subject with a change of more than 1 ppb in FeNO.

Lastly, subgroup analysis was performed on PPI users versus non-PPI users via spearman correlation. Correlation coefficients were similar in both groups (0.18 vs 0.21 for PPI nonusers vs PPI users).

Discussion

This study evaluates the relationship between FeNO and markers of EoE activity. Since peak esophageal eosinophil counts are the standard for diagnosing and monitoring disease activity, the correlation between FeNO level and

Table 1 Patient demographics

	Overall ($N = 50$)
Age	
Mean (SD)	43.6 (13.3)
Range	18–79
Female	
n (%)	23 (46%)
Seasonal allergies	
n (%)	19 (38%)
History of asthma	
Missing	1
n (%)	8 (16%)
History of eczema	
Missing	1
n (%)	3 (6%)
Any allergy	
n (%)	23 (46%)
History of heartburn	
n (%)	16 (32%)
History of regurgitation	
n (%)	7 (14%)
Rings	
n (%)	44 (88%)
Furrows	
n (%)	38 (76%)
White plaques	
Missing	1
n (%)	11 (22%)
Stricture	
n (%)	21 (42%)
Fragility	
n (%)	16 (32%)
PPI use	
n (%)	31 (62%)
Dysphagia	
Missing	1
n (%)	31 (63%)
FeNO	
Mean (SD)	29.8 (27.3)
Range	6–154
Peak eosinophil count	
Mean (SD)	32.5 (31.7)
Range	0–105

Demographics of the 50 patients who participated in study. The presence of dysphagia was determined by review of patients' clinic records near the time of testing. Patients taking any dose of a PPI were recorded in the "Taking PPI" category. Any Allergy is defined as any of Asthma, Eczema, or Seasonal Allergy

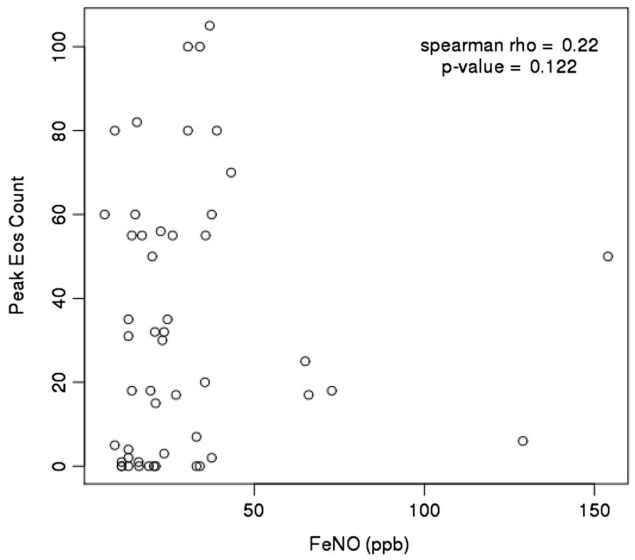

Fig. 1 Scatter plot of fractionated exhaled nitric oxide (ppb) versus peak esophageal eosinophil counts (eso/hpf). A weak spearman correlation was observed. The large majority of FeNO values were less than 50 ppb. The highest peak esophageal eosinophil counts corresponded to the FeNO < 50 ppb. The 5 of the 6 highest FeNO readings correlated with peak esophageal eosinophil counts less than 32.5, which was the mean value of the peak esophageal eosinophil counts

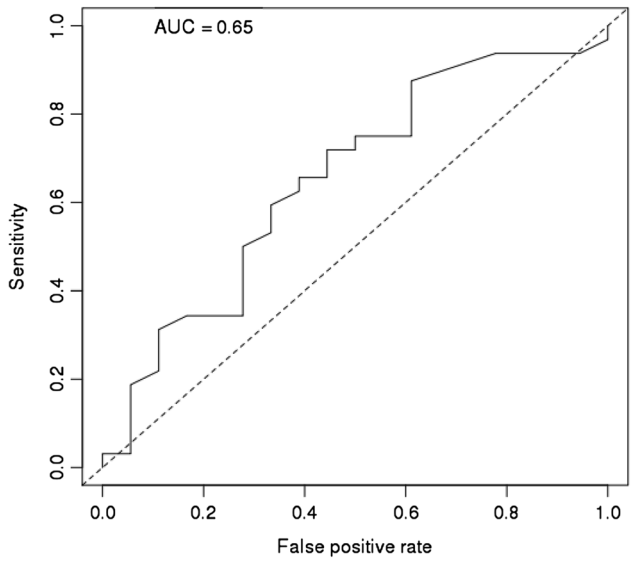

Fig. 2 Receiver operator curve for peak esophageal eosinophil counts (Peak Eosinophil ≥ 15 eos/hfp) versus fractionated exhaled nitric oxide. The area under the curve is 0.65 indicating limited ability to discern between Eoe activity using FeNO

peak esophageal eosinophil counts was the main focus of the study. The relationship for presence furrows was evaluated as a secondary marker because of its ability to correlate gross endoscopic findings to disease activity.

Spearman correlation of FeNO vs peak esophageal eosinophil count did not support a strong relationship ($r = 0.22$), with a wide range of peak eosinophil counts corresponding to similar FeNO levels.

In reviewing the ROC for FeNO versus peak eosinophil counts, the area under the curve of 0.65 indicates a weak ability for FeNO to discriminate between patients with and without eosinophil counts ≥ 15/hpf. Using a cut-off of FeNO ≥ 40 ppb would result in very few false positives, but many subjects with eosinophil count ≥ 15 would have false negative results. Elevated FeNO was a better predictor of elevated eosinophil counts than presence of furrows or dysphagia. Additionally, when using the ROC to evaluate different FeNO cut-off values, a sensitivity of 88% is achieved when the cut-off value decreased to > 13.5 ppb with resultant fall of specify to 39%. The high specificity of FeNO testing achieved at a cut-off of > 40 ppb indicates that the test may have utility in identifying a subset of patients with active disease to a high degree of certainty. This creates clinical benefit in this subset of patients by allowing a different way to monitor disease activity and avoid repeat endoscopy. However, only 6 of the 50 patients in the study met this level of FeNO, possibly indicating that the number of patients who could benefit from this testing would be relatively small.

Outside of asthma evaluation, the use of FeNO testing has been evaluated for use in other conditions, including eosinophilic atopic conditions, eczema, and IBD [15–23]. Furthermore, in IBD, nitric oxide levels found in serum, urine, aspirated colonic gas, and FeNO levels have shown to correlate with disease activity and steroid responsiveness [16–22]. In review of the literature, there are two studies that also evaluated the use of FeNO in patients with EoE. One study involving children showed high specificity (87%) and low sensitivity (50%) using a cut-off value of FeNO ≥ 15 ppb [11]. This study included 55 patients with only 14 having a FeNO level ≥ 15 ppb [11]. Of note 11 of these 14 were also noted to have decreased FEV1 or bronchodilator reversibly on spirometry indicating possible confounding undiagnosed asthma. Eighteen of the 55 were found to have EoE with only 9 of those having a FeNO level ≥ 15 ppb [11]. The other study was relatively small, including only 11 patients [12]. This study evaluated FeNO values before and after treatment with topical steroids. In post hoc analysis, all 11 patients were evaluated together and a decrease in both the median FeNO values and peak esophageal eosinophilic counts occurred with steroid treatment (20.3 ppb to 17.7 and 36 eos/hpf to 20) [12]. Only 5 of the 11 patients reached histological response that was defined as ≤ 7 eos/hpf and original subgroup analysis of patients classified as responders versus non-responders demonstrated no relationship between FeNO values and peak esophageal eosinophil counts in either group [12].

Strengths of our study include completion of the study at a center with eosinophilic esophagitis specialists in the

Table 2 Performance of various cut-offs for fractionated exhaled nitric oxide levels cut-offs (ppb) to predict elevated eosinophil level (≥ 15 eosinophils per hfp)

Cut-off performance	Cut-offs for exhaled nitric oxide				
	> 36.5	> 24	> 20.7	> 16.5	> 13.5
Sensitivity	0.25	0.50	0.66	0.75	0.88
Specificity	0.89	0.72	0.61	0.50	0.39
Positive predictive value	0.80	0.76	0.75	0.73	0.72
Negative predictive value	0.40	0.45	0.50	0.53	0.64

Note that positive and negative predictive values are dependent on sample prevalence and may not apply to other patient populations

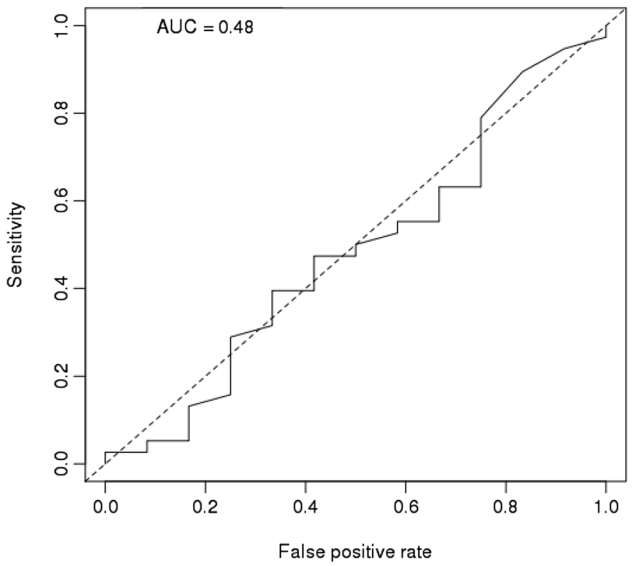

Fig. 3 The receiver operator curve for presence of furrows on endoscopy versus fractionated exhaled nitric oxide. The area under the curve is 0.48

clinic, endoscopy suite, and pathology lab. Additionally, there was limited exclusion criterion, with men and women recruited equally and a wide range of ages. This population did have a large proportion with allergies (any allergy, 46%) and 16% had history of asthma. Limitations to this study include the number of participants, the number of patients with FeNO levels above 40 ppb, and the retrospective method of identifying those with dysphagia from chart review. Only five patients were found to have FeNO levels greater than 50 ppb (the cut-off for indicating high likelihood of eosinophilic inflammation and corticosteroid responsiveness for asthma in adults) [10]. Four of these patients had history of asthma. This highlights a possible draw back to this form of testing in EoE since this population traditionally has an elevated prevalence of asthma. Lastly, there is always the concern of generalizability of results from a tertiary care medical center. However, over two-thirds of our patients were from MN or IA, suggesting the patients studied may reasonably represent of a primary or secondary referral practice.

The influence of atopic conditions on FeNO was not fully addressed in this study. FeNO levels are known to be influenced by systemic inflammation with increased consumption of nitric oxide in higher inflammatory states. The high number of patients with atopic conditions and resulting increased inflammation may therefore also be influencing the relationship seen between FeNO and eosinophil counts. Although, this might not be a likely mechanism since this hypothesized relationship is not seen in the testing of asthma, for which the test has known clinical utility [9, 10]. Although not demonstrated in our study, another possible cause for the weak correlation found maybe the high number of patients taking PPIs. Studies of PPIs in the cardiology field have shown PPI's capacity to inhibit the DDAH enzyme ex vivo [24]. The DDAH enzyme metabolizes ADMA, which is an inhibitor of nitric oxide synthase. This research has focused on vasodilation of blood vessels, but it does provide a possible mechanism for the low amounts of FeNO despite elevated eosinophil

Table 3 FeNO values compare to three clinical parameters

	FeNO ≤ 40 (ppb)	> 40 (ppb)	Sensitivity	Specificity	Pos pred value	Neg pred value
Eosinophil count < 15	17	1	0.16	0.94	0.83	0.39
Eosinophil count ≥ 15	27	5				
No furrows	10	2	0.11	0.83	0.67	0.23
Furrows	34	4				

These tables describe the relationship of elevated nitric oxide in relation to identifying each of the two measures (eosinophil counts and presences of furrows). Note that positive and negative predictive values are dependent on sample prevalence and may not apply to other patient populations

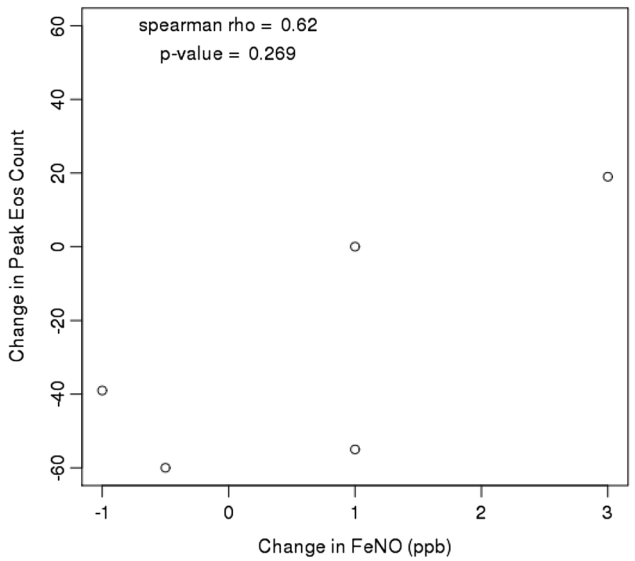

Fig. 4 Change in peak eosinophil counts on repeat endoscopy versus the change in FeNO for five patients on repeat testing. Results indicate a possible relationship within the individual for a change in eosinophil count resulting in a change in a FeNO level

levels seen in our study. On subgroup analysis of the non-PPI users in our patient population, there was no improvement in the spearman correlation of FeNO and peak eosinophil counts decreasing the likelihood of PPI use being a large contributing factor.

Though this study did not suggest a clinically useful association of FeNO and EoE activity for the majority of patients; there does appear to be a select group with very high FeNO values that may benefit given the high specificity for active histologic disease with FeNO values > 40 ppb. Future directions for research in this area could include a larger study that captured more patients with high levels of FeNO or a study powered to identify the role of the described possible confounders. This may elucidate who would benefit most from FeNO testing. Additionally, a larger trial looking at FeNO testing before and after treatment may show utility of the test in following individual patients for responsiveness. It is possible that the change in FeNO values for a specific patient with therapy may predict histologic response more reliably than the absolute values. Of note, our preliminary data with the patients who had received testing at two times points did not support this type of relationship.

FeNO levels seen in our clinical testing did not show a strong relationship to histologic esophageal eosinophilia and at this time cannot be recommended for clinical use. Future investigations might focus on the change in FeNO levels for an individual patient undergoing therapy.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent Informed consent was obtained from all individual participants included in the study.

References

1. Dellon ES, Liacouras CA. Advances in clinical management of eosinophilic esophagitis. Gastroenterology. 2014;147(6):1238–54. https://doi.org/10.1053/j.gastro.2014.07.055.
2. Gonsalves N, Yang GY, Doerfler B, Ritz S, Ditto AM, Hirano I. Elimination diet effectively treats eosinophilic esophagitis in adults; food reintroduction identifies causative factors. Gastroenterology. 2012;142(7):1451–9. https://doi.org/10.1053/j.gastro.2012.03.001.
3. Lucendo AJ, Arias A, Gonzalez-Cervera J, Yague-Compadre JL, Guagnozzi D, Angueira T, Jimenez-Contreras S, Gonzalez-Castillo S, Rodriguez-Domingez B, De Rezende LC, Tenias JM. Empiric 6-food elimination diet induced and maintained prolonged remission in patients with adult eosinophilic esophagitis: a prospective study on the food cause of the disease. J Allergy Clin Immunol. 2013;131(3):797–804. https://doi.org/10.1016/j.jaci.2012.12.664.
4. Spergel JM, Andrews T, Brown-Whitehorn TF, Beausoleil JL, Liacouras CA. Treatment of eosinophilic esophagitis with specific food elimination diet directed by a combination of skin prick and patch tests. Ann Allergy Asthma Immunol. 2005;95(4):336–43. https://doi.org/10.1016/s1081-1206(10)61151-9.
5. Henderson CJ, Abonia JP, King EC, Putnam PE, Collins MH, Franciosi JP, Rothenberg ME. Comparative dietary therapy effectiveness in remission of pediatric eosinophilic esophagitis. J Allergy Clin Immunol. 2012;129(6):1570–8. https://doi.org/10.1016/j.jaci.2012.03.023.
6. Philpott H, Nandurkar S, Royce SG, Gibson PR. Ultrathin unsedated transnasal gastroscopy in monitoring eosinophilic esophagitis. J Gastroenterol Hepatol. 2016;31(3):590–4. https://doi.org/10.1111/jgh.13173.
7. Katzka DA, Geno DM, Ravi A, Smyrk TC, Lao-Sirieix P, Miremadi A, Debiram I, O'Donovan M, Kita H, Kephart GM, Kryzer LA, Camilleri M, Alexander JA, Fitzgerald RC. Accuracy, safety, and tolerability of tissue collection by Cytosponge vs endoscopy for evaluation of eosinophilic esophagitis. Clin Gastroenterol Hepatol. 2015;13(1):77–83. https://doi.org/10.1016/j.cgh.2014.06.026.
8. Dellon ES, Rusin S, Gebhart JH, Covey S, Higgins LL, Beitia R, Speck O, Woodward K, Woosley JT, Shaheen NJ. Utility of a

noninvasive serum biomarker panel for diagnosis and monitoring of eosinophilic esophagitis: a prospective study. Am J Gastroenterol. 2015;110(6):821–7. https://doi.org/10.1038/ajg.2015.57.
9. Mummadi SR, Hahn PY. Update on exhaled nitric oxide in clinical practice. Chest. 2016;149(5):1340–4. https://doi.org/10.1016/j.chest.2015.11.020.
10. Dweik RA, Boggs PB, Erzurum SC, Irvin CG, Leigh MW, Lundberg JO, Olin AC, Plummer AL, Taylor DR. An official ATS clinical practice guideline: interpretation of exhaled nitric oxide levels (FENO) for clinical applications. Am J Respir Crit Care Med. 2011;184(5):602–15. https://doi.org/10.1164/rccm.9120-11ST.
11. Lanz MJ, Guerrero RA, Gonzalez-Vallina R. Measurement of exhaled nitric oxide in the evaluation for eosinophilic esophagitis in children. Ann Allergy Asthma Immunol. 2012;109(1):81–2. https://doi.org/10.1016/j.anai.2012.05.009.
12. Leung J, Nguyen-Traxler A, Lee EM, Yip JS, Weinstock JV, Chan WW, Ngo P, Weinstein BJ, Bonis PA. Assessment of fractionated exhaled nitric oxide as a biomarker for the treatment of eosinophilic esophagitis. Allergy Asthma Proc. 2012;33(6):519–24. https://doi.org/10.2500/aap.2012.33.3606.
13. Hirano I, Moy N, Heckman MG, Thomas CS, Gonsalves N, Achem SR. Endoscopic assessment of the oesophageal features of eosinophilic oesophagitis: validation of a novel classification and grading system. Gut. 2013;62(4):489–95. https://doi.org/10.1136/gutjnl-2011-301817.
14. R core team (2017) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
15. Olin AC, Rosengren A, Thelle DS, Lissner L, Bake B, Toren K. Height, age, and atopy are associated with fraction of exhaled nitric oxide in a large adult general population sample. Chest. 2006;130(5):1319–25. https://doi.org/10.1378/chest.130.5.1319.
16. Scott M, Raza A, Karmaus W, Mitchell F, Grundy J, Kurukulaaratchy RJ, Arshad SH, Roberts G. Influence of atopy and asthma on exhaled nitric oxide in an unselected birth cohort study. Thorax. 2010;65(3):258–62. https://doi.org/10.1136/thx.2009.125443.
17. Rees DC, Satsangi J, Cornelissen PL, Travis SP, White J, Jewell DP. Are serum concentrations of nitric oxide metabolites useful for predicting the clinical outcome of severe ulcerative colitis? Eur J Gastroenterol Hepatol. 1995;7(3):227–30.
18. Oudkerk Pool M, Bouma G, Visser JJ, Kolkman JJ, Tran DD, Meuwissen SG, Pena AS. Serum nitrate levels in ulcerative colitis and Crohn's disease. Scand J Gastroenterol. 1995;30(8):784–8.
19. Kimura H, Miura S, Shigematsu T, Ohkubo N, Tsuzuki Y, Kurose I, Higuchi H, Akiba Y, Hokari R, Hirokawa M, Serizawa H, Ishii H. Increased nitric oxide production and inducible nitric oxide synthase activity in colonic mucosa of patients with active ulcerative colitis and Crohn's disease. Dig Dis Sci. 1997;42(5):1047–54.
20. Rachmilewitz D, Eliakim R, Ackerman Z, Karmeli F. Direct determination of colonic nitric oxide level–a sensitive marker of disease activity in ulcerative colitis. Am J Gastroenterol. 1998;93(3):409–12. https://doi.org/10.1111/j.1572-0241.1998.00409.x.
21. Goggins MG, Shah SA, Goh J, Cherukuri A, Weir DG, Kelleher D, Mahmud N. Increased urinary nitrite, a marker of nitric oxide, in active inflammatory bowel disease. Mediat Inflamm. 2001;10(2):69–73. https://doi.org/10.1080/09629350120054536.
22. Koek GH, Verleden GM, Evenepoel P, Rutgeerts P. Activity related increase of exhaled nitric oxide in Crohn's disease and ulcerative colitis: a manifestation of systemic involvement? Respir Med. 2002;96(7):530–5.
23. Cross RK, Wilson KT. Nitric oxide in inflammatory bowel disease. Inflamm Bowel Dis. 2003;9(3):179–89.
24. Ghebremariam YT, LePendu P, Lee JC, Erlanson DA, Slaviero A, Shah NH, Leiper J, Cooke JP. Unexpected effect of proton pump inhibitors: elevation of the cardiovascular risk factor asymmetric dimethylarginine. Circulation. 2013;128(8):845–53. https://doi.org/10.1161/circulationaha.113.003602.

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