Use of Exhaled Nitric Oxide Measurement to Identify a Reactive, at-Risk Phenotype among Patients with Asthma

Raed A. Dweik[1–3], Ronald L. Sorkness[3], Sally Wenzel[3], Jeffrey Hammel[1], Douglas Curran-Everett[3], Suzy A. A. Comhair[1,3], Eugene Bleecker[3], William Busse[3], William J. Calhoun[3], Mario Castro[3], Kian Fan Chung[3], Elliot Israel[3], Nizar Jarjour[3], Wendy Moore[3], Stephen Peters[3], Gerald Teague[3], Benjamin Gaston[3], and Serpil C. Erzurum[1–3]; for the National Heart, Lung, and Blood Institute Severe Asthma Research Program*

[1]Department of Pathobiology/Lerner Research Institute and [2]Department of Pulmonary, Allergy, and Critical Care Medicine/Respiratory Institute, Cleveland Clinic, Cleveland, Ohio; and [3]Severe Asthma Research Program, National Heart, Lung, and Blood Institute, Bethesda, Maryland

Rationale: **Exhaled nitric oxide (F_{ENO}) is a biomarker of airway inflammation in mild to moderate asthma. However, whether F_{ENO} levels are informative regarding airway inflammation in patients with severe asthma, who are refractory to conventional treatment, is unknown. Here, we hypothesized that classification of severe asthma based on airway inflammation as defined by F_{ENO} levels would identify a more reactive, at-risk asthma phenotype.**

Methods: **F_{ENO} and major features of asthma, including airway inflammation, airflow limitation, hyperinflation, hyperresponsiveness, and atopy, were determined in 446 individuals with various degrees of asthma severity (175 severe, 271 nonsevere) and 49 healthy subjects enrolled in the Severe Asthma Research Program.**

Measurements and Main Results: **F_{ENO} levels were similar among patients with severe and nonsevere asthma. The proportion of individuals with high F_{ENO} levels (>35 ppb) was the same (40%) among groups despite greater corticosteroid therapy in severe asthma. All patients with asthma and high F_{ENO} had more airway reactivity (maximal reversal in response to bronchodilator administration and by methacholine challenge), more evidence of allergic airway inflammation (sputum eosinophils), more evidence of atopy (positive skin tests, higher serum IgE and blood eosinophils), and more hyperinflation, but decreased awareness of their symptoms. High F_{ENO} identified those patients with severe asthma characterized by the greatest airflow obstruction and hyperinflation and most frequent use of emergency care.**

Conclusions: **Grouping of asthma by F_{ENO} provides an independent classification of asthma severity, and among patients with severe asthma identifies the most reactive and worrisome asthma phenotype.**

Keywords: nitric oxide; severe asthma; phenotype; airway reactivity; exhaled breath

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Exhaled nitric oxide (F_{ENO}) is a biomarker of airway inflammation in mild to moderate asthma. However, whether F_{ENO} levels are informative regarding airway inflammation in patients with severe asthma, who are refractory to conventional treatment, is unknown.

What This Study Adds to the Field

Here, we demonstrate that grouping of asthma by F_{ENO} provides an independent classification of asthma severity, and among patients with severe asthma identifies the most reactive and worrisome asthma phenotype.

Despite progress that has been made in the understanding and treatment of mild and moderate asthma, severe asthma is poorly understood, refractory to established treatments, and accounts for a high proportion of the adverse financial impact, morbidity, and mortality of asthma in the United States (1–4). The underlying reasons why certain individuals with asthma have severe, refractory disease are poorly defined. Although sputum eosinophils have been shown to predict acute exacerbations in asthma (5, 6), sputum induction is not easy to do or widely available. Thus, there is a need for a noninvasive, easy-to-perform test to monitor patients with severe asthma and predict acute and often life-threatening asthma exacerbations, and thus allow for determination of whether or not therapy is adequate (1–4). As a free radical that reacts with oxidants and antioxidants, nitric oxide (NO) in exhaled breath (F_{ENO}) reflects the redox state of the airway and has been proposed as a marker of airway inflammation and guide for antiinflammatory therapy in asthma (7). High levels of F_{ENO} are well documented in nonsevere asthma (8–21) and decrease in response to treatment with corticosteroids (22–27). However, measures of F_{ENO} in 50 patients with severe asthma in the European multicenter study of chronic severe asthma suggest that F_{ENO} levels of patients with severe asthma, who are refractory to conventional treatments, may not be suppressed by corticosteroids (28). Although the mean F_{ENO} levels of patients with severe asthma were similar to those of patients with nonsevere asthma, 22 (44%) of the subjects with severe asthma who were receiving high-dose oral corticosteroids had threefold higher F_{ENO} than those receiving inhaled corticosteroids, which suggested that a substantial subpopulation of patients with severe asthma had persistent airway inflammation and possible relative corticosteroid resistance.

In this study, we hypothesized that classification of severe asthma based on airway inflammation as defined by F_{ENO} levels would identify a more severe asthma phenotype. The present study was designed to assess alterations of F_{ENO} in patients with severe asthma as compared with patients with nonsevere asthma and healthy control subjects, and the relationship between F_{ENO}

(*Received in original form May 8, 2009; accepted in final form February 1, 2010*)

Supported by HL69170, AI70649, HL04265, HL68863, HL69116, HL69174, HL69167, HL069155, HL69130, HL69149, HL69170, HL069349, ACRN U10HL74225, P01/U01HL67663, and P01/U01 HL67663; by a Third Frontier grant (TECH09-003) from the Ohio Department of Development; and by M01 RR018390 from the National Center for Research Resources.

* A list of contributors can be found at the end of the article.

Correspondence and requests for reprints should be addressed to Raed A. Dweik, M.D., Department of Pulmonary, Allergy, and Critical Care Medicine/Respiratory Institute, Desk A-90, Cleveland Clinic, 9500 Euclid Avenue, Cleveland, OH 44195. E-mail: dweikr@ccf.org

This article has an online supplement, which is accessible from this issue's table of contents at www.atsjournals.org

Am J Respir Crit Care Med Vol 181. pp 1033–1041, 2010
Originally Published in Press as DOI: 10.1164/rccm.200905-0695OC on February 4, 2010
Internet address: www.atsjournals.org

and asthma severity, airflow limitation, hyperinflation, hyperresponsiveness, and atopy. Although the average F_{ENO} levels in severe and nonsevere asthma were previously reported to be similar (29), when asthma was classified on the basis of F_{ENO} levels, a distinct asthma phenotype emerged. In general, patients with asthma and high F_{ENO} levels tended to be younger and diagnosed with asthma at a younger age. They were more likely to be atopic and to have evidence of airway inflammation. Furthermore, patients with severe asthma and high F_{ENO} levels had the greatest airway reactivity, the most hyperinflation, and the least awareness of their asthma symptoms. The findings provide evidence that F_{ENO} levels are informative for classification of severe asthma phenotypes and allow identification of a particularly worrisome subgroup of patients with severe asthma. Some of the results of these studies have been previously reported in the form of an abstract (30).

METHODS

Detailed methods and statistical analyses are provided in the online supplement. A brief description is provided here.

Subject Enrollment and Characterization

All subjects were recruited by centers participating in the Severe Asthma Research Program (SARP) and gave written informed consent by signing a consent document approved by the institutional review board at the enrolling center and the SARP Data Safety and Monitoring Board (DSMB). All subjects were screened by history, physical examination, spirometry (before and after two puffs of inhaled albuterol), methacholine provocation, and allergy prick skin testing to a standard panel of aeroallergens. Subjects were nonsmokers, and classified as healthy control subjects if they were free of respiratory symptoms, had normal baseline spirometry, a negative methacholine challenge test, and nitric oxide level less than 50 ppb. Asthma was defined according to the National Asthma Education and Prevention Program guidelines, which include episodic respiratory symptoms, reversible airflow obstruction (documentation of variability of FEV_1 and/or FVC by 12% and 200 cm^3 either spontaneously or after two puffs of inhaled albuterol), and/or a positive methacholine challenge test (4). Severe asthma was based on the definition used by the proceedings of the American Thoracic Society Workshop on Refractory Asthma (2).

Lung Function

Spirometry was performed with an automated spirometer, consistent with American Thoracic Society (ATS) standards (31). Plethysmographic lung volumes, including total lung capacity (TLC) and residual volume (RV), were measured in 62 subjects with severe asthma and 53 subjects with nonsevere asthma, using methods conforming to ATS guidelines (32), and recorded as the percentage of predicted values obtained with the equations of Stocks and Quanjer (33), with adjustments for African Americans per ATS recommendations (34).

Atopy

Allergy skin testing was done once on each subject during the study. Skin prick testing to 14 common allergens was performed at all SARP sites with the Multi-Test II (Lincoln Diagnostics, Inc., Decatur, IL). Blood was collected for measurement of total serum IgE and a complete blood count.

Exhaled NO (F_{ENO})

All SARP centers performed online and/or offline NO measurements according to the standards published by the ATS (35). Online F_{ENO} values were used in all data analyses in this article. NO levels were measured online by chemiluminescence at a constant expiratory flow (50 ml/s) in all participating centers. The analyzers were calibrated in accordance with the manufacturer's instructions. Because spirometry can affect the F_{ENO} levels, exhaled gases were collected before spirometry, if completed on the same day. On the basis of data suggesting poor asthma control when F_{ENO} is more than 35 ppb (7), we evaluated clinical characteristics of asthma populations in subgroups of high (>35 ppb) and low (<35 ppb) NO. The rationale for selecting 35 ppb as a cutoff point for high and low NO was based on the published literature (7) and analysis of the data collected in this study. In addition to the published literature, Figure 1 provides the rationale for selecting 35 ppb as a cutoff point for high and low F_{ENO} that is the basis for all data analyses in this study. Relevant variables (as outlined in Table E1 in the online supplement) in the database were analyzed on the basis of receiver operator characteristic (ROC) curves with F_{ENO} as a continuous variable. The cutoff point for each variable was determined on the basis of these ROC curves. Figure 1 represents the frequency distribution of all these cutoff points. The median of all cutoff points for the variables (both categorical and continuous) that showed a significant relationship with F_{ENO} was 37 ppb. This provided support for the validity of our selection of 35 ppb as the cutoff point between high and low F_{ENO}.

Total NO Reaction Products

NO reaction products (NOx) in serum samples were measured by an amperometric NO sensor in combination with acidified iodide for the detection of NO derived from total nitrite and nitrate after cadmium/copper-mediated reduction of nitrate to nitrite (ISO-NOP, Nitralyzer II; World Precision Instruments, Sarasota, FL) (36).

Statistical Analyses

Categorical data were summarized as frequencies, and statistical comparisons for categorical variables were performed using Fisher's exact test. Subgroup comparisons within NO level or asthma severity were performed using appropriate contrasts from a logistic regression model including NO level, asthma severity, and their interaction as independent variables. Continuous variables were summarized using

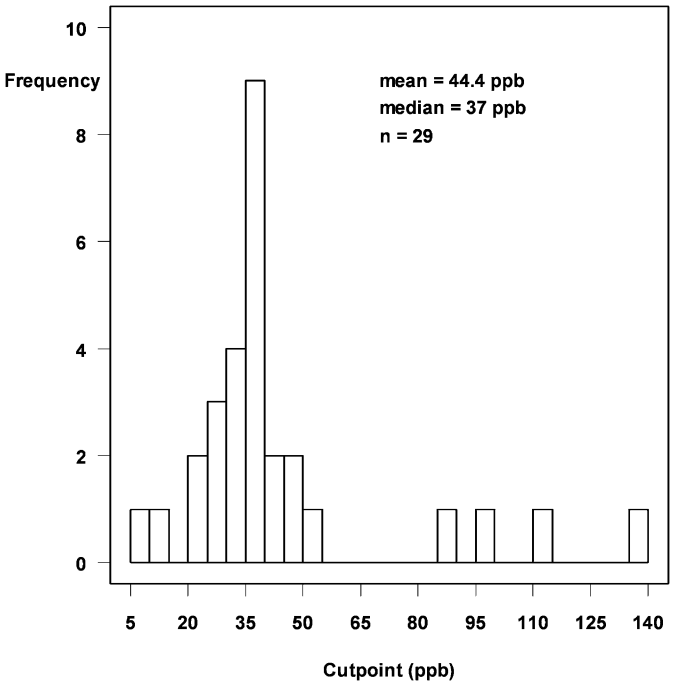

Figure 1. Relevant variables (as outlined in Table E1 in the online supplement) in the database were analyzed on the basis of receiver operator characteristic (ROC) curves with F_{ENO} as a continuous variable. The cutoff point for each variable was determined on the basis of these ROC curves. Shown here is the frequency distribution of all these cutoff points. The median of all cutoff points for the variables (both categorical and continuous) that showed a significant relationship with F_{ENO} was 37 ppb. This provided support for the validity of our selection of 35 ppb as the cutoff point between high and low F_{ENO}.

the sample size, mean, and standard deviation, and alternatively using the median and interquartile range for variables with skewed distributions. Associations between NO levels and other variables were assessed by linear regression for $F_{E_{NO}}$ as a continuous variable and multiple logistic regression for $F_{E_{NO}}$ (high or low) as categorical variables. Multiple logistic regression modeling is described in more detail in RESULTS. All tests and model fitting were performed with the JMP statistical program, version 5.0 (SAS Institute Inc., Cary, NC) and R version 2.4.1 (www.R-project.org) (37). Models for $F_{E_{NO}}$ as a continuous outcome in a linear regression model and as a dichotomous outcome classified as high or low in a logistic regression model were created. For multivariate analyses and modeling, parsimonious selection of independent variables was performed to avoid confounding that would render the estimated associations with the outcome as noninterpretable or misleading. Similarly, a logistic regression model for which the $F_{E_{NO}}$ outcome would be classified as high or low had to be parsimonious to be mathematically stable.

RESULTS

Characterization of Study Population

Online $F_{E_{NO}}$ levels were measured in 495 individuals enrolled in the Severe Asthma Research Program (SARP). Baseline characteristics are shown in Table 1. On average, healthy control subjects and patients with nonsevere asthma were younger than patients with severe asthma ($P < 0.05$) (Table 1). As expected, lung functions were lower in patients with severe asthma than in patients with nonsevere asthma or healthy control subjects (Table 1). The detailed clinical description of individuals in the SARP data set was previously published (29). The SARP population included in the current study does not overlap with the SARP subpopulation of children with offline NO values published previously (38).

NO in Asthma

NO levels were higher in patients with asthma as compared with control subjects, but there was no significant difference in average $F_{E_{NO}}$ between severe and nonsevere asthma ($F_{E_{NO}}$ [ppb]: control, 17 ± 9; nonsevere, 43 ± 42; severe, 42 ± 41; $P = 0.01$) (Table 1). The proportion of individuals with high $F_{E_{NO}}$ was the same in severe and nonsevere asthma (nonsevere, 109/271 [40%]; severe, 70/175 [40%]).

The High-NO Phenotype in Asthma

There were equal proportions of patients with severe and nonsevere asthma in the low- and high-$F_{E_{NO}}$ groups. In general, patients with asthma and high $F_{E_{NO}}$ demonstrated several distinct characteristics when compared with patients with asthma and low $F_{E_{NO}}$. Demographically, patients with asthma and high $F_{E_{NO}}$ were younger (age, yr [mean ± SD]: low $F_{E_{NO}}$, 38 ± 12; high $F_{E_{NO}}$, 36 ± 13; $P = 0.03$) and diagnosed with asthma at a younger age (age, yr [mean ± SD]: low $F_{E_{NO}}$, 16 ± 13; high $F_{E_{NO}}$, 14 ± 14; $P = 0.05$) and less likely to be female (female [% of population]: low $F_{E_{NO}}$, 70%; high $F_{E_{NO}}$, 60%; $P = 0.02$).

On pulmonary function testing, the high- and low-$F_{E_{NO}}$ groups had similar baseline FEV_1 and FVC, but the FEV_1/FVC ratio (% predicted) was lower in high $F_{E_{NO}}$, indicating increased airflow limitation in this group. The high-$F_{E_{NO}}$ group also had more airway reactivity as shown by greater FEV_1 reversibility after maximal bronchodilation and lower PC_{20} (provocative concentration of methacholine causing a 20% fall in FEV_1). They had more hyperinflation with a higher total lung capacity (TLC), a higher residual lung volume (RV), and a higher RV/TLC ratio (Table 2).

High-$F_{E_{NO}}$ patients with asthma, whether severe or nonsevere, were more likely to be atopic as shown by more positive skin tests (number of positive skin tests [mean ± SD]: low $F_{E_{NO}}$, 3.4 ± 3; high $F_{E_{NO}}$, 4.2 ± 3; $P = 0.004$), higher serum IgE level (serum IgE [mean ± SD]: low $F_{E_{NO}}$, 219 ± 366; high $F_{E_{NO}}$, 340 ± 402; $P = 0.0001$), and higher blood eosinophils (% blood eosinophils [mean ± SD]: low $F_{E_{NO}}$, 3.4 ± 3.7; high $F_{E_{NO}}$, 5.1 ± 3.9; $P = 0.0001$). They also had more eosinophils in sputum (% sputum eosinophils [mean ± SD]: low $F_{E_{NO}}$, 3 ± 7; high $F_{E_{NO}}$, 13 ± 23; $P = 0.0001$), suggesting more evidence of allergic airway inflammation. Interestingly, patients with asthma and high $F_{E_{NO}}$ levels were less likely to have seen a physician in the last 12 months (%: low $F_{E_{NO}}$, 72%; high $F_{E_{NO}}$, 63%; $P = 0.04$),

TABLE 1. DEMOGRAPHICS, PULMONARY FUNCTION, EXHALED NITRIC OXIDE LEVELS, AND CORTICOSTEROIDS USAGE FOR ALL SUBJECTS

	Control Subjects (*n = 49*)	n	Nonsevere Asthma (*n = 271*)	n	Severe Asthma (*n = 175*)	n
Mean age, yr*	32 ± 11	49	34 ± 12	271	41 ± 13	175
Baseline %FEV_1*	101 ± 15	49	83 ± 16	271	58 ± 20	175
Maximal %FEV_1*	108 ± 15	37	93 ± 15	256	76 ± 20	168
% FVC*	103 ± 11	49	94 ± 14	271	80 ± 19	176
% FEV_1/FVC*, ppb	97 ± 7	49	88 ± 12	271	77 ± 14	175
$F_{E_{NO}}$, ppb	17 ± 9	49	43 ± 42	271	42 ± 41	175
Median, IQR*	14 (11–19)	49	27 (17–55)	271	27 (17–52)	175
Sex (male), no.	13	49	86	271	65	176
Race, C/AA/AI/A/NH/O/U/R/MR	40/5/0/2/0/0/0/0/2	49	172/80/0/4/0/8/1/0/6	271	116/44/0/7/0/2/0/0/6	175
Corticosteroids						
Inhaled, %*	0	49	64%	271	100%	175
Oral, %*	0	49	3%	271	44%	175
Injected, %	0	49	0%	271	3%	175
Serum IgE levels*	58 ± 87	45	267 ± 380	239	318 ± 730	147
Median, IQR*	32 (10–60)	45	145 (60–330)	239	124 (40–320)	147
BAL eosinophils, %*	0.2 ± 0.7	21	1.1 ± 0.4	73	1.9 ± 0.5	49
Median, IQR*	0 (0–0.4)	21	0.3 (0–1.2)	73	0.5 (0–1.5)	49
Blood eosinophils, %*	2.3 ± 1	45	4.1 ± 3	252	4.1 ± 5	168
Median, IQR*	2 (1–2.85)	45	3.7 (2–5)	252	3 (1.5–5)	168

Definition of abbreviations: A = Asian; AA =African American; AI = American Indian or Alaska native; BAL = bronchoalveolar lavage; C = Caucasian; $F_{E_{NO}}$ = exhaled nitric oxide level; IQR = interquartile range; MR = multiple races; n = number of individuals with available data; NH = Native Hawaiian; O = other; R = refused; U = uncertain.

* Fisher's, analysis of variance, or Kruskal-Wallis $P < 0.05$ among three groups.

TABLE 2. PULMONARY FUNCTION BY EXHALED NITRIC OXIDE

Characteristic	Low F_{ENO} (≤35 ppb)	n	High F_{ENO} (>35 ppb)	n	*P* Value
Baseline FVC, % predicted	85 ± 18	267	87 ± 19	179	0.20
Maximal FVC, % of predicted	93 ± 16	253	100 ± 15	170	<0.001
Baseline FEV_1, % predicted	74 ± 20	267	73 ± 23	179	0.80
Maximal FEV_1, % of predicted	85 ± 20	253	90 ± 18	170	0.005
FEV_1/FVC ratio, % of predicted	86 ± 14	267	81 ± 14	179	<0.001
Maximal FEV_1 reversal, %	14 ± 16	253	20 ± 17	170	
Median (IQR)*	10 (5–18)	253	16 (8–26)	170	<0.001
PC_{20}	4.3 ± 6	203	1.7 ± 3	123	
Median (IQR)*	1.8 (0.5–4.9)	203	0.7 (0.3–1.6)	123	<0.001
TLC, % predicted	106 ± 12	88	115 ± 14	43	0.002
FRC, % predicted	101 ± 24	84	119 ± 30	40	<0.001
FRC/TLC, % predicted	95 ± 18	84	103 ± 16	40	0.008
RV, % predicted	124 ± 42	88	153 ± 57	43	0.003
RV/TLC, % predicted	111 ± 30	88	126 ± 40	42	0.03

Definition of abbreviations: IQR = interquartile range; PC_{20} = provocative concentration of methacholine causing a 20% fall in FEV_1; RV = reserve volume; TLC = total lung capacity.

* Wilcoxon rank sum *P* values reported rather than *t* test.

but more likely to have been in the emergency room (%: low F_{ENO}, 66%; high F_{ENO}, 73%; $P = 0.05$) over the same time period, or admitted to the intensive care unit in the past (%: low F_{ENO}, 16%; high F_{ENO}, 25%; $P = 0.02$).

Patients with asthma and low NO levels were more likely to be overweight (body mass index, kg/m^2 [mean ± SD]: low F_{ENO}, 31 ± 9; high F_{ENO}, 28 ± 8; $P = 0.002$), have systemic hypertension (%: low F_{ENO}, 16%; high F_{ENO}, 8%; $P = 0.05$), and be on treatment for diabetes (%: low F_{ENO}, 40%; high F_{ENO}, 11%; $P = 0.01$).

Characterizing the High-F_{ENO} Phenotype in Severe Asthma

In patients with severe asthma, high F_{ENO} levels identified a phenotype that appeared to be the most severe of all groups, including low-F_{ENO} severe asthma, and high- or low-F_{ENO} nonsevere asthma groups. Individuals with severe asthma and high F_{ENO} levels tended to share several characteristics. They had the greatest airway reactivity of any group defined as the magnitude of FEV_1 reversal after maximal bronchodilation and by PC_{20}. They had the greatest degree of airflow limitation and the most hyperinflation (Table 3). They also had high numbers of eosinophils in the sputum (Table 4). Emergency room use and intensive care unit admissions were greatest in this group (Table 3). In contrast to F_{ENO}, NO metabolites (NOx) in serum were higher in all patients with severe asthma as a group in comparison with patients with nonsevere asthma (NOx, μM: nonsevere, 36 ± 23; severe, 42 ± 24; $P = 0.0009$) and were unrelated to F_{ENO} levels ($R = 0.002$; $P = 0.5$). Serum NOx was not related to clinical characteristics such as lung function or atopy (all $P > 0.2$).

Characterizing the High-F_{ENO} Phenotype in Nonsevere Asthma

In patients with nonsevere asthma, high F_{ENO} similarly identified a more severe subgroup. In fact, the patients with nonsevere asthma and high F_{ENO} shared more similarities with

TABLE 3. PULMONARY FUNCTION AND EXHALED NITRIC OXIDE LEVEL BY SEVERITY

Characteristic	Severe Asthma, Low F_{ENO}	n	Nonsevere Asthma, Low F_{ENO}	n	Severe Asthma, High F_{ENO}	n	Nonsevere Asthma, High F_{ENO}	n	Low F_{ENO}: Severe vs. Nonsevere Asthma	High F_{ENO}: Severe vs. Nonsevere Asthma	Severe Asthma: Low vs. High F_{ENO}	Nonsevere Asthma: Low vs. High F_{ENO}
Baseline FVC, % predicted	75 ± 18	105	92 ± 15	162	75 ± 21	70	95 ± 14	109	<0.001	<0.001	0.97	0.13
Maximal FVC, % predicted	88 ± 17	101	97 ± 14	153	95 ± 18	67	103 ± 12	103	<0.001	<0.001	0.004	<0.001
Baseline FEV_1, % predicted	60 ± 19	105	83 ± 16	162	56 ± 22	70	83 ± 17	109	<0.001	<0.001	0.24	0.76
Maximal FEV_1, % predicted	74 ± 20	101	91 ± 15	153	80 ± 19	67	96 ± 15	103	<0.001	<0.001	0.009	0.04
FEV_1/FVC ratio, % predicted	79 ± 15	105	90 ± 11	162	74 ± 14	70	86 ± 12	109	<0.001	<0.001	0.011	0.03
Maximal FEV_1 reversal, %	18 ± 23	101	11 ± 9	153	23 ± 19	67	17 ± 15	103				
Median (IQR)*	14 (6–22)	101	8 (5–14)	153	21 (9–29)	67	13 (7–22)	103	0.002	0.01	0.005	<0.001
PC_{20}	3.9 ± 6	53	4.4 ± 6	149	1.5 ± 3	27	1.7 ± 3	96				
Median (IQR)*	1 (0.2–4.5)	53	2 (0.6–5)	149	0.6 (0.2–1.7)	27	0.7 (0.3–1.6)	96	0.10	0.40	0.01	<0.001
TLC, % predicted	107 ± 13	41	104 ± 12	31	117 ± 17	21	112 ± 10	22	0.55	0.28	0.006	0.05
FRC, % predicted	103 ± 27	38	96 ± 21	31	124 ± 34	18	115 ± 26	22	0.40	0.36	0.005	0.01
FRC/TLC, % predicted	96 ± 21	38	92 ± 14	31	104 ± 16	18	102 ± 17	22	0.43	0.84	0.10	0.03
RV, % predicted	143 ± 44	41	109 ± 34	31	176 ± 58	21	131 ± 47	22	0.004	0.001	0.005	0.11
RV/TLC, % predicted	128 ± 30	41	99 ± 21	31	141 ± 41	20	111 ± 33	22	<0.001	0.002	0.12	0.21
ER in past 12 mo	38%	105	12%	161	53%	70	18%	109	<0.001	<0.001	0.05	0.14
Ever had an ICU admission due to asthma	34%	105	5%	162	44%	70	12%	108	<0.001	<0.001	0.26	0.04
BMI	32 ± 8	103	30 ± 9	162	30 ± 8	66	28 ± 7	109	0.08	0.13	0.08	0.016

Definition of abbreviations: BMI = body mass index; ER = emergency room; ICU = intensive care unit; IQR = interquartile range; RV = reserve volume; TLC = total lung capacity.

High F_{ENO} defined as >35 ppb, and low F_{ENO} as ≤35 ppb.

* Wilcoxon rank sum *P* values reported rather than those based on contrasts from analysis of variance.

TABLE 4. INFLAMMATORY CELLS IN BLOOD, BRONCHOALVEOLAR LAVAGE, AND SPUTUM BY SEVERITY AND EXHALED NITRIC OXIDE LEVELS

Characteristic	Severe Asthma, Low F_{ENO}	n	Nonsevere Asthma, Low F_{ENO}	n	Severe Asthma, High F_{ENO}	n	Nonsevere Asthma, High F_{ENO}	n	Low F_{ENO}: Severe vs. Nonsevere Asthma	High F_{ENO}: Severe vs. Nonsevere Asthma	Severe Asthma: Low vs. High F_{ENO}	Nonsevere Asthma: Low vs. High F_{ENO}
Inflammatory cells in blood												
Total WBC	7.8 ± 3	103	6.8 ± 2	153	8 ± 2	64	6.5 ± 2	104	0.001	<0.001	0.76	0.19
Monocytes, %	5.7 ± 2	103	6.4 ± 2	152	6.0 ± 3	63	6.8 ± 2	104	0.23	0.83	0.06	0.16
Neutrophils, %	62 ± 12	103	58 ± 10	152	63 ± 15	64	55 ± 10	104	0.27	0.46	0.81	0.63
Lymphocytes, %	28 ± 11	103	32 ± 9	152	25 ± 10	64	33 ± 9	104	0.16	<0.001	0.27	0.11
Eosinophils, %	3.8 ± 5.2	103	3.1 ± 2.1	152	4.7 ± 3.9	64	5.3 ± 3.9	104	0.20	0.27	0.13	<0.001
Basophils, %	0.4 ± 0.5	103	0.6 ± 0.5	144	0.5 ± 0.5	64	0.5 ± 0.6	104	0.005	0.36	0.34	0.60
Inflammatory cells in BAL												
BAL total cells	8 ± 6	27	9 ± 8	48	4.8 ± 3	22	8.9 ± 6	27	0.55	0.02	0.09	0.95
BAL macrophages, %	91 ± 10	27	90 ± 16	47	82 ± 24	22	81 ± 26	26	0.74	0.85	0.10	0.07
BAL neutrophils, %	3.0 ± 4	27	2.1 ± 4	47	2.6 ± 4	22	3.2 ± 7	26	0.42	0.62	0.72	0.34
BAL lymphocytes, %	3.8 ± 3	27	5.3 ± 6	47	8.9 ± 13	22	6.4 ± 6	26	0.40	0.23	0.02	0.56
BAL eosinophils, %	1.9 ± 6	27	0.8 ± 2.2	47	1.8 ± 3	22	1.5 ± 2.4	26	0.18	0.77	0.93	0.39
Inflammatory cells in sputum												
Total cells, millions	4.1 ± 7	61	3.0 ± 4	117	3.3 ± 5	34	2.4 ± 3	80	0.24	0.98	0.52	0.76
Total WBCs, millions	3.1 ± 6	61	2.1 ± 4	117	2.5 ± 5	34	2.4 ± 9	79	0.27	0.92	0.61	0.76
Viability of WBCs, %	61 ± 24	62	63 ± 21	117	58 ± 23	34	62 ± 22	79	0.48	0.30	0.53	0.83
Bronchial epithelial cells, %	4.8 ± 6	62	2.9 ± 5	117	2.4 ± 3	34	3.7 ± 4	79	0.02	0.21	0.03	0.30
Sputum macrophages, %	52 ± 25	56	56 ± 26	99	39 ± 32	28	63 ± 26	66	0.38	<0.001	0.05	0.11
Sputum lymphocytes, %	4.2 ± 5	56	2.5 ± 3	99	3.1 ± 6	28	2.7 ± 2	66	0.008	0.64	0.20	0.82
Sputum neutrophils, %	40 ± 25	56	39 ± 26	99	32 ± 30	28	27 ± 23	66	0.79	0.36	0.23	0.006
Sputum eosinophils, %	3 ± 5	56	2.2 ± 4	99	25 ± 33	28	7 ± 13	66	0.38	<0.001	<0.001	0.02

Definition of abbreviation: BAL = bronchoalveolar lavage; WBCs = white blood cells.

patients with severe asthma and high F_{ENO} than with patients with nonsevere asthma and low F_{ENO}. For instance, the nonsevere group with high F_{ENO} had more airway reactivity defined by the magnitude of FEV_1 reversal after maximal bronchodilation and by PC_{20}, and significantly more airflow limitation and hyperinflation than patients with nonsevere asthma and low F_{ENO} levels. They also had more eosinophilic inflammation (Table 4) and more intensive care unit (although not emergency room) admissions. These individuals were the thinnest among all groups (Table 3).

F_{ENO} and Lung Volumes

TLC increased linearly with increased air trapping as measured by elevated ratio of RV to TLC. In addition, there was an independent additive increase in TLC in subjects with higher F_{ENO} ($P = 0.0005$ for F_{ENO} effect, $P < 0.0001$ for RV/TLC effect; analysis of covariance). There was no effect of the designated severe or nonsevere asthma grouping ($P > 0.9$) on TLC independent of air trapping and F_{ENO} effects within each of the severity groups. This indicates that air trapping and F_{ENO} are independent determinants for lung hyperinflation in asthma.

F_{ENO} and Use of Corticosteroids and Other Medications

The greater reactivity in the high-F_{ENO} asthma subgroups suggested that these patients had greater airway inflammation and/or less antiinflammatory therapy. All patients with severe asthma in this study were by definition receiving some form of corticosteroids (2). There was no difference in the use of inhaled corticosteroid or leukotriene modifiers among patients with asthma and high or low F_{ENO}, but more patients in the high-F_{ENO} group were taking oral corticosteroids (Table 5). Individuals with high F_{ENO} were more likely to be taking theophylline (Table 5). When corticosteroid use was further analyzed by asthma severity in addition to F_{ENO} levels, again there was no significant difference in inhaled corticosteroid use between the high- and low-F_{ENO} groups regardless of severity. The group with high F_{ENO} and severe asthma had the highest proportion of oral corticosteroid use (percent oral corticosteroid use: severe asthma–low F_{ENO}, 37%; nonsevere asthma–low F_{ENO}, 1%; severe asthma–high F_{ENO}, 56%; nonsevere asthma–high F_{ENO}, 5%; $P = 0.01$). Whereas only a small number of individuals were taking theophylline, patients with severe asthma and high F_{ENO} levels were much more likely to be taking daily theophylline than members of any of the other groups (percent theophylline use: severe asthma–low F_{ENO}, 13%; nonsevere asthma–low F_{ENO}, 1%; severe asthma–high F_{ENO}, 29%; nonsevere asthma–high F_{ENO}, 3%; $P = 0.01$). Thus, the finding of high F_{ENO} in the severe and nonsevere asthma subgroups was likely not due to less corticosteroid therapy than in the low-F_{ENO} subgroups. Multivariate analyses and modeling for determinants of F_{ENO} did not indicate an influence of corticosteroid use on F_{ENO} levels (Table 6).

DISCUSSION

This study provides evidence that subclassification by F_{ENO} defines asthma phenotypes independent of current definitions

TABLE 5. MEDICATION USE BY EXHALED NITRIC OXIDE LEVELS

Characteristic	Low F_{ENO} (≤35 ppb)	n	High F_{ENO} (>35 ppb)	n	*P* Value*
Inhaled corticosteroids	28%	267	28%	179	0.9
Oral corticosteroids	15%	267	25%	179	0.01
Injectable corticosteroids	2.2%	267	3.9%	179	0.3
Inhaled corticosteroids and β-agonist	57%	267	50%	179	0.1
Total β-agonists	91%	267	91%	179	0.9
Total long-acting β-agonist	65%	267	59%	179	0.2
Total inhaled corticosteroids	73%	267	70%	179	0.5
Total other corticosteroids	16%	267	25%	179	0.02
Leukotriene modifiers	29%	267	31%	179	0.6
Theophylline	6%	267	13%	179	0.01

* Fisher's exact test *P* values.

for asthma severity. Patients with asthma who have high F_{ENO} levels share several characteristics regardless of their asthma severity as it is currently defined. Patients with asthma and high F_{ENO} are younger and diagnosed with asthma at a younger age. They are atopic and have more eosinophilic airway inflammation, more airway reactivity, more airflow limitation, and more hyperinflation. The fact that patients with high F_{ENO} were more likely to have gone to an emergency room or admitted to an ICU over the past 12 months also suggests that they may be less aware of early symptoms of their disease. Within the severe asthma group of subjects, high F_{ENO} identifies a severe asthma phenotype that has the greatest eosinophilic airway inflammation, the most severe airflow limitation, and uses emergent care most often.

NO is produced by nitric oxide synthases (NOS), including constitutive (neuronal, or type 1, and endothelial, or type 3) and inducible (type 2) enzymes, all isoforms of which are present in the lung (39, 40). Abnormalities in NOS1 and NOS2 genotype and expression are associated with asthma and increased NOS activity is associated with increased F_{ENO} levels (41–43). F_{ENO} represents a balance between NO production and consumption (10). In particular, NO is rapidly consumed by reaction with superoxide. There is direct evidence that more severe obstruction in asthma is associated with increased spontaneous and stimulus-induced generation of superoxide by inflammatory cells in the airway (44).

The independent association of elevated F_{ENO} with increased TLC is a novel finding, and suggests that there is an inflammatory component affecting lung mechanics that is separate from the air-trapping mechanism. Increased TLC has been associated with acute asthma exacerbation and with poorly controlled chronic asthma, and many of these subjects exhibit a decrease in TLC after therapy with bronchodilators and corticosteroids (45–47). Further studies are needed to determine the nature of the interaction between NO and TLC in asthma, but the current study shows that F_{ENO} is associated with hyperinflation in asthma. Furthermore, F_{ENO} in severe asthma might reflect airway-remodeling processes (48, 49). Because many of the variables that are related to NO are also related to severity, relationships between variables and F_{ENO} were also evaluated within severity group by multivariate analyses. Multivariate analyses and modeling confirmed most of the associations suggested by the univariate analyses and revealed new findings as well. For instance, the relationship between F_{ENO} and markers of atopy and eosinophilic inflammation was confirmed in nonsevere asthma, which suggests a strong dependence of F_{ENO} on these variables. However, the multivariate significance of factors that influence F_{ENO} in all asthma was driven primarily by the nonsevere asthma group. Most features did not significantly influence F_{ENO} in severe asthma. This suggests that features other than those evaluated in this study may be determinants of high F_{ENO} levels in severe asthma.

There are several possible explanations for the presence of high F_{ENO} in patients with severe asthma. Because the F_{ENO} levels of patients with nonsevere asthma decrease in response to corticosteroid therapy (22–27), the greater corticosteroid use in the severe asthma group would be expected to result in low levels of F_{ENO}. In this context, one possible explanation for the high F_{ENO} may be inadequate corticosteroid therapy. However, high F_{ENO} levels in patients with severe asthma on high-dose oral or injectable corticosteroids are difficult to explain on this basis. Noncompliance with therapy is possible, but this explanation needs to invoke that patients with severe asthma with high F_{ENO} are less compliant than patients with severe asthma with low F_{ENO}, even though they report similar corticosteroid use. Importantly, high F_{ENO} was not related to corticosteroid therapy in any analysis. Furthermore, the current ATS workshop criteria are meant to distinguish patients with severe asthma, but not to define those with nonsevere asthma. This may partially explain why the patients with high F_{ENO} have similar characteristics in both patient groups. Taken together with previous studies (1, 3, 23, 28, 50–54), the high-F_{ENO} severe asthma group may have relative resistance to corticosteroid therapy. In support of this, individuals with severe asthma and high F_{ENO} are more likely to be taking theophylline. Given the current clinical practice of reserving theophylline use in asthma for individuals not responding to traditional therapies, greater use of daily theophylline in the high-F_{ENO} group is another indirect marker of more difficult to treat, less corticosteroid-responsive asthma.

Analyses of the characteristics of individuals with low F_{ENO} and severe asthma in this study also provide new information about asthma phenotypes. F_{ENO} levels reflect a balance between the rates of NO production and its consumption, which is largely related to oxidant–NO reactions (10, 55, 56). Thus, low levels of F_{ENO} in asthma may be related to less NO synthesis or greater oxidative consumption. Mechanisms that

TABLE 6. RESULTS OF MULTIVARIATE LOGISTIC REGRESSION ANALYSIS WITH HIGH EXHALED NITRIC OXIDE (>35 ppb) AS THE OUTCOME

	All Asthma (*n* = 335)		Nonsevere Asthma (*n* = 210)		Severe Asthma (*n* = 125)	
Characteristic	Odds Ratio (95% CI)	*P* Value	Odds Ratio (95% CI)	*P* Value	Odds Ratio (95% CI)	*P* Value
Sex	0.99 (0.55–1.61)	0.96	1.04 (0.50–2.05)	0.90	1.11 (0.40–2.70)	0.82
Age	0.99 (0.97–1.01)	0.57	0.97 (0.95–1.00)	0.058	1.02 (0.99–1.05)	0.22
BMI	0.99 (0.95–1.02)	0.40	1.02 (0.98–1.07)	0.36	0.94 (0.89–1.00)	0.06
Activity score	1.42 (1.10–1.80)	0.005	1.47 (1.08–2.03)	0.02	1.52 (0.89–2.31)	0.08
Wheezing	1.14 (0.92–1.38)	0.17	1.17 (0.89–1.50)	0.24	1.19 (0.80–1.68)	0.33
Log maximal FEV_1 reversal	1.19 (1.01–1.41)	0.03	1.22 (0.99–1.51)	0.06	1.29 (0.91–1.73)	0.1
Log IgE	1.88 (1.19–3.01)	0.007	2.71 (1.38–5.36)	0.004	1.52 (0.74–3.20)	0.25
Log percent eosinophils in the blood	1.35 (1.11–1.62)	0.002	1.15 (1.14–1.93)	0.003	1.31 (0.97–1.78)	0.07
Total ICS	0.74 (0.40–1.39)	0.34	0.68 (0.34–1.40)	0.30	3.14 (0.07–95.54)	0.53
Total other CS	1.34 (0.66–3.20)	0.41	2.05 (0.18–22.45)	0.55	1.76 (0.68–5.15)	0.23
Theophylline use	2.92 (1.23–7.54)	0.02	9.0 (0.71–102.62)	0.08	2.9 (1.13–9.30)	0.04
Seen a doctor in the last 12 mo	0.58 (0.31–1.09)	0.09	0.75 (0.35–1.61)	0.46	0.5 (0.12–2.89)	0.38
Visited ER in the last 12 mo	2.33 (1.20–4.69)	0.01	2.65 (0.87–7.90)	0.09	2.5 (1.08–7.69)	0.05

Definition of abbreviations: BMI = body mass index; CI = confidence interval; CS = corticosteroids; ER = emergency room; ICS = inhaled corticosteroids.
n = number of individuals who had a complete set of all variables to run the model.

affect NO production include factors that modify NOS enzyme activity or expression, alter nonenzymatic release of NO from storage pools, or change the denitrifying organisms that colonize the upper airway (57–61). However, the end products of NO consumption are greater in severe asthma than in nonsevere asthma, which suggests that total NO production is greater in severe asthma but may not be reflected by levels in the exhaled breath because of greater oxidative consumption. In support of this concept, features of metabolic syndrome, which is characterized by oxidative stress and abnormalities of NO metabolism, were observed in the low-F_{ENO} asthma group, that is, higher body mass index, hypertension, and diabetes.

Strengths of this study include the large cohort, the well-characterized population, and the prospective and standardized method of data collection. The main imitation of this study is also the fact that it is a cohort and not a randomized controlled trial. F_{ENO} levels were analyzed in a cross-sectional fashion and not based on or before and after an intervention. Certain variables such as compliance with therapy could not be completely accounted for and verified.

Clinical asthma phenotypes have been recognized for some time (51, 62), but quantitative biomarkers have not been previously identified in severe asthma (51). This has limited the clear discrimination and understanding of severe asthma. Detailed and quantitative phenotypes will further our understanding of the pathobiology and genetics that contribute to severe asthma genesis (51). Although the current definition of asthma severity is useful for clinical research, it is cumbersome to use and impractical for the busy office setting. The availability of an easy-to-measure, noninvasive marker would greatly simplify and improve severe asthma management (63). The current findings suggest that evaluation of multiple quantitative biologic markers, such as F_{ENO}, circulating NO reaction products, and sputum eosinophils, may provide a cumulative index for definition of asthma severity in the future. Here, F_{ENO} is identified as a biomarker that distinguishes a group of patients with severe airflow obstruction, hyperreactivity, hyperinflation, and persistent airway inflammation. Although the retrospective nature of our analysis has precluded us from determining whether F_{ENO} could predict future risk of exacerbations in asthma, its correlation with ER visits and hospital and ICU admission suggests a great potential for F_{ENO} in identifying those patients with the most severe disease in clinical practice. Prospective studies would be helpful in confirming this fact that is suggested by our findings and to ascertain the determinants of the high-F_{ENO} phenotype in patients with severe asthma, who are refractory to therapy.

The Severe Asthma Research Program (SARP) is a multicenter asthma research group funded by the NHLBI and consisting of the following contributors (Steering Committee members are marked with an asterisk): Brigham and Women's Hospital, Boston, MA—Elliot Israel,* Bruce D. Levy, Gautham Marigowda; Cleveland Clinic, Cleveland, OH—Serpil C. Erzurum,* Raed A. Dweik, Suzy A. A. Comhair, Marcelle Baaklini, Daniel Laskowski, Jacqueline Pyle; Emory University, Atlanta, GA—W. Gerald Teague,* Anne M. Fitzpatrick, Eric Hunter; Imperial College School of Medicine, London, UK—Kian F. Chung,* Mark Hew, Alfonso Torrego, Sally Meah, Mun Lim; National Jewish Medical and Research Center, Denver, CO—Sally E. Wenzel,* Diane Rhodes; University of Pittsburgh, Pittsburgh, PA—William J. Calhoun,* Melissa P. Clark, Renee Folger, Rebecca Z. Wade; Bill T. Ameredes, Dori Smith; University of Virginia, Charlottesville, VA—Benjamin Gaston,* Peter Urban; University of Wisconsin, Madison, WI—William W. Busse,* Nizar Jarjour, Erin Billmeyer, Ronald L. Sorkness, Cheri Swenson, Gina Crisafi; Wake Forest University, Winston-Salem, NC—Eugene R. Bleecker,* Deborah Meyers, Wendy Moore, Stephen Peters, Annette Hastie, Gregory Hawkins, Jeffrey Krings, Regina Smith; Washington University in St. Louis, St. Louis, MO—Mario Castro,* Leonard Bacharier, Iftikhar Hussain, Jaime Tarsi; Data Coordinating Center, Denver, CO—James R. Murphy,* Douglas Curran-Everett; NHLBI, Bethesda, MD—Patricia Noel.

Conflict of Interest Statement: R.A.D. received more than $100,001 from Actelion, more than $100,001 from Gilead, and more than $100,001 from Novartis in industry-sponsored grants. R.L.S. received up to $1,000 from Synairgen in consultancy fees, and more than $100,001 from Modus Biological Membranes in industry-sponsored grants. The spouse/life partner of R.L.S. received $5,001–$10,000 from GlaxoSmithKline in advisory board fees, and $5,001–$10,000 from GlaxoSmithKline and $1,001–$5,000 from AstraZeneca in lecture fees. S.W. received $1,001–$5,000 from Amgen, $10,001–$50,000 from GlaxoSmithKline, $1,001–$5,000 from Wyeth, and $1,001–$5,000 from Genentech in consultancy fees; $1,001–$5,000 from GlaxoSmithKline, $10,001–$50,000 from Amira, $10,001–$50,000 from Epigenesis, and $5,001–$10,000 from Novartis in advisory board fees; $1,001–$5,000 from Critical Therapeutics and $1,001–$5,000 from Genentech in lecture fees; $50,001–$100,000 from GlaxoSmithKline, $5,001–$10,000 from Amgen, $10,001–$50,000 from MedImmune, $50,001–$100,000 from Ception, and $10,001–$50,000 from Aerovance in industry-sponsored grants; and holds stock ownership or options in Amira and Epigenesis. J.H. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. D.C.-E. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. S.A.A.C. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. E.R.B. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. W.B. received up to $1,000 from Alexion, $1,001–$5,000 from AstraZeneca, $1,001–$5,000 from Boehringer Ingelheim, up to $1,000 from Dainippon–Sumitomo, up to $1,000 from Funxional Therapeutics, Ltd., $1,001–$5,000 from Novartis, and $1,001–$5,000 from TEVA in consultancy fees; $1,001–$5,000 from Altair, $1,001–$5,000 from Amgen, $1,001–$5,000 from Abbott Laboratories, $1,001–$5,000 from Asthmatx, Inc., $1,001–$5,000 from Bristol–Meyers Squib, $1,001–$5,000 from Centocor, up to $1,000 from Genentech, up to $1,000 from GlaxoSmithKline, $1,001–$5,000 from Merck, $1,001–$5,000 from Schering Plough, $1,001–$5,000 from Pfizer, and $1,001–$5,000 from Wyeth in advisory board fees; $1,001–$5,000 from Merck in lecture fees; and $10,001–$50,000 from Novartis, $5,001–$10,000 from Centocor, more than $100,001 from GlaxoSmithKline, more than $100,001 from MedImmune, and $50,001–$100,000 from Ception in industry–sponsored grants. W.J.C. received $10,001–$50,000 from AstraZeneca and $5,001–$10,000 from Sepracor in consultancy fees, and $5,001–$10,000 from Merck in advisory board fees. M.C. received $10,001–$50,000 from Asthmatx, $1,001–$5,000 from Schering, $1,001–$5,000 from Electrocore, and $1,001–$5,000 from BMS in consultancy fees; $5,001–$10,000 Genentech in advisory board fees; $50,001–$100,000 from AstraZeneca, $10,001–$50,000 from Boehringer Ingelheim, $10,001–$50,000 from Pfizer, $5,001–$10,000 from Genentech, and $5,001–$10,000 from Merck in lecture fees; more than $100,001 from Asthmatx, more than $100,001 from Amgen, more than $100,001 from Centocor, more than $100,001 from Ception, and more than $100,001 from GlaxoSmithKline in industry-sponsored grants; $1,001–$5,000 from Elsevier in royalties; and $10,001–$50,000 from Pfizer, more than $100,001 from Genentech, more than $100,001 from MedImmune, more than $100,001 from Merck, and more than $100,001 from Novartis in contracted research. K.F.C. received $1,001–$5,000 from Gilead for ad hoc consulting; $1,001–$5,000 from GlaxoSmithKline, $1,001–$5,000 from Chiesi, $1,001–$5,000 from Merck, and $1,001–$5,000 from Boehringer Ingelheim for serving on an advisory board; $1,001–$5,000 from AstraZeneca, $1,001–$5,000 from GlaxoSmithKline, and $1,001–$5,000 from Chiesi in nonpromotional lecture fees; $10,001–$50,000 from Novartis for a phase 3 clinical trial, $1,001–$5,000 from Schering Plough for a phase 3 clinical trial, $1,001–$5,000 from AstraZeneca for phase 3 research, and $50,001–$100,000 from GlaxoSmithKline (collaborative) in industry-sponsored grants; and holds a patent from Imperial Innovations on use of p38 MAPK inhibitors to reverse corticosteroid resistance in airway disease. E.I. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. N.J. received $10,001–$50,000 from Asthmatx for review of CT scans, concept discussions, and advisory group meetings; $1,001–$5,000 from GlaxoSmithKline and $1,001–$5,000 from Genentech in advisory board fees; $1,001–$5,000 from Merck and Co. in lecture fees; and more than $100,001 from GlaxoSmithKline and more than $100,001 from Genentech in industry-sponsored grants. W.M. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. S.P. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. G.T. received $10,001–$50,000 from Merck and Co. and up to $1,000 from Aerocrine in lecture fees. The B.G. laboratory received up to $1,000 from Galleon in consultancy fees, more than $100,001 from Galleon in industry-sponsored grants, and more than $100,001 from the NIH in sponsored grants; $1,001–$5,000 from Williams and Connelly as an expert witness; holds patents from Galleon (for control of breathing: therapy), Respiratory Research (for breath condensate analysis), Airbase (for asthma treatment), and N30 (for asthma and CF treatment); and has received $5,001–$10,000 from Respiratory Research in royalties. S.C.E. received more than $100,001 from Asthmatx as an investigator in industry-sponsored grants.

Acknowledgment: The authors thank A. Janocha for the performance of NOx analyses.

References

1. Busse WW, Banks-Schlegel S, Wenzel SE. Pathophysiology of severe asthma. *J Allergy Clin Immunol* 2000;106:1033–1042.

2. American Thoracic Society. Proceedings of the ATS workshop on refractory asthma: current understanding, recommendations, and unanswered questions. *Am J Respir Crit Care Med* 2000;162:2341–2351.
3. Wenzel SE, Schwartz LB, Langmack EL, Halliday JL, Trudeau JB, Gibbs RL, Chu HW. Evidence that severe asthma can be divided pathologically into two inflammatory subtypes with distinct physiologic and clinical characteristics. *Am J Respir Crit Care Med* 1999;160: 1001–1008.
4. National Asthma Education and Prevention Program, National Heart, Lung, and Blood Institute. Guidelines for the diagnosis and the management of asthma: expert panel report II. Bethesda, MD: National Institutes of Health; 1997.
5. Green RH, Brightling CE, McKenna S, Hargadon B, Parker D, Bradding P, Wardlaw AJ, Pavord ID. Asthma exacerbations and sputum eosinophil counts: a randomised controlled trial. *Lancet* 2002; 360:1715–1721.
6. Jayaram L, Pizzichini MM, Cook RJ, Boulet LP, Lemiere C, Pizzichini E, Cartier A, Hussack P, Goldsmith CH, Laviolette M, *et al.* Determining asthma treatment by monitoring sputum cell counts: effect on exacerbations. *Eur Respir J* 2006;27:483–494.
7. Smith AD, Cowan JO, Brassett KP, Herbison GP, Taylor DR. Use of exhaled nitric oxide measurements to guide treatment in chronic asthma. *N Engl J Med* 2005;352:2163–2173.
8. Olin AC, Bake B, Toren K. Fraction of exhaled nitric oxide at 50 mL/s: reference values for adult lifelong never-smokers. *Chest* 2007;131: 1852–1856.
9. Smith AD, Cowan JO, Filsell S, McLachlan C, Monti-Sheehan G, Jackson P, Taylor DR. Diagnosing asthma: comparisons between exhaled nitric oxide measurements and conventional tests. *Am J Respir Crit Care Med* 2004;169:473–478.
10. Dweik RA, Comhair SA, Gaston B, Thunnissen FB, Farver C, Thomassen MJ, Kavuru M, Hammel J, Abu-Soud HM, Erzurum SC. NO chemical events in the human airway during the immediate and late antigen-induced asthmatic response. *Proc Natl Acad Sci USA* 2001;98:2622–2627.
11. Guo FH, Comhair SA, Zheng S, Dweik RA, Eissa NT, Thomassen MJ, Calhoun W, Erzurum SC. Molecular mechanisms of increased nitric oxide (NO) in asthma: evidence for transcriptional and post-translational regulation of NO synthesis. *J Immunol* 2000;164:5970–5980.
12. Kharitonov SA, Yates D, Robbins RA, Logan-Sinclair R, Shinebourne EA, Barnes PJ. Increased nitric oxide in exhaled air of asthmatic patients. *Lancet* 1994;343:133–135.
13. Massaro AF, Mehta S, Lilly CM, Kobzik L, Reilly JJ, Drazen JM. Elevated nitric oxide concentrations in isolated lower airway gas of asthmatic subjects. *Am J Respir Crit Care Med* 1996;153:1510–1514.
14. Persson MG, Zetterstrom O, Agrenius V, Ihre E, Gustafsson LE. Single-breath nitric oxide measurements in asthmatic patients and smokers. *Lancet* 1994;343:146–147.
15. Thomassen MJ, Raychaudhuri B, Dweik RA, Farver C, Buhrow L, Malur A, Connors MJ, Drazba J, Hammel J, Erzurum SC, *et al.* Nitric oxide regulation of asthmatic airway inflammation with segmental allergen challenge. *J Allergy Clin Immunol* 1999;104:1174–1182.
16. Khatri SB, Hammel J, Kavuru MS, Erzurum SC, Dweik RA. Temporal association of nitric oxide levels and airflow in asthma after whole lung allergen challenge. *J Appl Physiol* 2003;95:436–440, discussion 435.
17. Deykin A, Halpern O, Massaro AF, Drazen JM, Israel E. Expired nitric oxide after bronchoprovocation and repeated spirometry in patients with asthma. *Am J Respir Crit Care Med* 1998;157:769–775.
18. Massaro AF, Gaston B, Kita D, Fanta C, Stamler JS, Drazen JM. Expired nitric oxide levels during treatment of acute asthma. *Am J Respir Crit Care Med* 1995;152:800–803.
19. Delgado-Corcoran C, Kissoon N, Murphy SP, Duckworth LJ. Exhaled nitric oxide reflects asthma severity and asthma control. *Pediatr Crit Care Med* 2004;5:48–52.
20. Payne DN, Adcock IM, Wilson NM, Oates T, Scallan M, Bush A. Relationship between exhaled nitric oxide and mucosal eosinophilic inflammation in children with difficult asthma, after treatment with oral prednisolone. *Am J Respir Crit Care Med* 2001;164: 1376–1381.
21. Silkoff PE, Sylvester JT, Zamel N, Permutt S. Airway nitric oxide diffusion in asthma: role in pulmonary function and bronchial responsiveness. *Am J Respir Crit Care Med* 2000;161:1218–1228.
22. Silkoff PE, McClean P, Spino M, Erlich L, Slutsky AS, Zamel N. Dose–response relationship and reproducibility of the fall in exhaled nitric oxide after inhaled beclomethasone dipropionate therapy in asthma patients. *Chest* 2001;119:1322–1328.
23. ten Brinke A, Zwinderman AH, Sterk PJ, Rabe KF, Bel EH. "Refractory" eosinophilic airway inflammation in severe asthma: effect of parenteral corticosteroids. *Am J Respir Crit Care Med* 2004;170:601–605.
24. Lim S, Jatakanon A, John M, Gilbey T, O'Connor BJ, Chung KF, Barnes PJ. Effect of inhaled budesonide on lung function and airway inflammation: assessment by various inflammatory markers in mild asthma. *Am J Respir Crit Care Med* 1999;159:22–30.
25. Dupont LJ, Rochette F, Demedts MG, Verleden GM. Exhaled nitric oxide correlates with airway hyperresponsiveness in steroid-naive patients with mild asthma. *Am J Respir Crit Care Med* 1998;157:894–898.
26. Mattes J, Storm van's Gravesande K, Reining U, Alving K, Ihorst G, Henschen M, Kuehr J. NO in exhaled air is correlated with markers of eosinophilic airway inflammation in corticosteroid-dependent childhood asthma. *Eur Respir J* 1999;13:1391–1395.
27. Kharitonov SA, Yates DH, Barnes PJ. Inhaled glucocorticoids decrease nitric oxide in exhaled air of asthmatic patients. *Am J Respir Crit Care Med* 1996;153:454–457.
28. European Network for Understanding Mechanisms of Severe Asthma. The ENFUMOSA cross-sectional European multicentre study of the clinical phenotype of chronic severe asthma. *Eur Respir J* 2003;22: 470–477.
29. Moore WC, Bleecker ER, Curran-Everett D, Erzurum SC, Ameredes BT, Bacharier L, Calhoun WJ, Castro M, Chung KF, Clark MP, *et al.* Characterization of the severe asthma phenotype by the National Heart, Lung, and Blood Institute's Severe Asthma Research Program. *J Allergy Clin Immunol* 2007;119:405–413.
30. Dweik RA, Erzurum SC; Severe Asthma Research Program. Low levels of exhaled nitric oxide in severe asthma [abstract]. *Proc Am Thorac Soc* 2005;2:A244.
31. American Thoracic Society. Standardization of spirometry, 1994 update. *Am J Respir Crit Care Med* 1995;152:1107–1136.
32. Wanger J, Clausen JL, Coates A, Pedersen OF, Brusasco V, Burgos F, Casaburi R, Crapo R, Enright P, van der Grinten CP, *et al.* Standardisation of the measurement of lung volumes. *Eur Respir J* 2005;26: 511–522.
33. Stocks J, Quanjer PH. Reference values for residual volume, functional residual capacity and total lung capacity: ATS workshop on lung volume measurements [official statement of the European Respiratory Society]. *Eur Respir J* 1995;8:492–506.
34. American Thoracic Society. Lung function testing: selection of reference values and interpretative strategies. *Am Rev Respir Dis* 1991;144: 1202–1218.
35. American Thoracic Society, European Respiratory Society. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. *Am J Respir Crit Care Med* 2005;171:912–930.
36. Xu W, Kaneko FT, Zheng S, Comhair SA, Janocha AJ, Goggans T, Thunnissen FB, Farver C, Hazen SL, Jennings C, *et al.* Increased arginase II and decreased NO synthesis in endothelial cells of patients with pulmonary arterial hypertension. *FASEB J* 2004;18:1746–1748.
37. R Development Core Team. R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2006.
38. Fitzpatrick AM, Gaston BM, Erzurum SC, Teague WG. Features of severe asthma in school-age children: atopy and increased exhaled nitric oxide. *J Allergy Clin Immunol* 2006;118:1218–1225.
39. Kobzik L, Bredt DS, Lowenstein CJ, Drazen J, Gaston B, Sugarbaker D, Stamler JS. Nitric oxide synthase in human and rat lung: immunocytochemical and histochemical localization. *Am J Respir Cell Mol Biol* 1993;9:371–377.
40. Dweik RA, Laskowski D, Abu-Soud HM, Kaneko F, Hutte R, Stuehr DJ, Erzurum SC. Nitric oxide synthesis in the lung: regulation by oxygen through a kinetic mechanism. *J Clin Invest* 1998;101:660–666.
41. De Sanctis GT, MacLean JA, Hamada K, Mehta S, Scott JA, Jiao A, Yandava CN, Kobzik L, Wolyniec WW, Fabian AJ, *et al.* Contribution of nitric oxide synthases 1, 2, and 3 to airway hyperresponsiveness and inflammation in a murine model of asthma. *J Exp Med* 1999; 189:1621–1630.
42. Wechsler ME, Grasemann H, Deykin A, Silverman EK, Yandava CN, Israel E, Wand M, Drazen JM. Exhaled nitric oxide in patients with asthma: association with NOS1 genotype. *Am J Respir Crit Care Med* 2000;162:2043–2047.

43. Grasemann H, Yandava CN, Storm van's Gravesande K, Deykin A, Pillari A, Ma J, Sonna LA, Lilly C, Stampfer MJ, Israel E, *et al.* A neuronal NO synthase (NOS1) gene polymorphism is associated with asthma. *Biochem Biophys Res Commun* 2000;272:391–394.
44. Calhoun WJ, Reed HE, Moest DR, Stevens CA. Enhanced superoxide production by alveolar macrophages and air-space cells, airway inflammation, and alveolar macrophage density changes after segmental antigen bronchoprovocation in allergic subjects. *Am Rev Respir Dis* 1992;145:317–325.
45. Blackie SP, al-Majed S, Staples CA, Hilliam C, Pare PD. Changes in total lung capacity during acute spontaneous asthma. *Am Rev Respir Dis* 1990;142:79–83.
46. Gold WM, Kaufman HS, Nadel JA. Elastic recoil of the lungs in chronic asthmatic patients before and after therapy. *J Appl Physiol* 1967;23: 433–438.
47. Woolcock AJ, Read J. The static elastic properties of the lungs in asthma. *Am Rev Respir Dis* 1968;98:788–794.
48. van Veen IH, Ten Brinke A, Sterk PJ, Sont JK, Gauw SA, Rabe KF, Bel EH. Exhaled nitric oxide predicts lung function decline in difficult-to-treat asthma. *Eur Respir J* 2008;32:344–349.
49. Sugiura H, Komaki Y, Koarai A, Ichinose M. Nitrative stress in refractory asthma. *J Allergy Clin Immunol* 2008;121:355–360.
50. Wenzel SE. Phenotypes in asthma: useful guides for therapy, distinct biological processes, or both? *Am J Respir Crit Care Med* 2004;170:579–580.
51. Wenzel SE. Asthma: defining of the persistent adult phenotypes. *Lancet* 2006;368:804–813.
52. Chanez P, Wenzel SE, Anderson GP, Anto JM, Bel EH, Boulet LP, Brightling CE, Busse WW, Castro M, Dahlen B, *et al.* Severe asthma in adults: what are the important questions? *J Allergy Clin Immunol* 2007;119:1337–1348.
53. in't Veen JC, Smits HH, Hiemstra PS, Zwinderman AE, Sterk PJ, Bel EH. Lung function and sputum characteristics of patients with severe asthma during an induced exacerbation by double-blind steroid withdrawal. *Am J Respir Crit Care Med* 1999;160:93–99.
54. ten Brinke A, Zwinderman AH, Sterk PJ, Rabe KF, Bel EH. Factors associated with persistent airflow limitation in severe asthma. *Am J Respir Crit Care Med* 2001;164:744–748.
55. Comhair SA, Ricci KS, Arroliga M, Lara AR, Dweik RA, Song W, Hazen SL, Bleecker ER, Busse WW, Chung KF, *et al.* Correlation of systemic superoxide dismutase deficiency to airflow obstruction in asthma. *Am J Respir Crit Care Med* 2005;172:306–313.
56. Comhair SA, Bhathena PR, Dweik RA, Kavuru M, Erzurum SC. Rapid loss of superoxide dismutase activity during antigen-induced asthmatic response. *Lancet* 2000;355:624.
57. Ricciardolo FL, Gaston B, Hunt J. Acid stress in the pathology of asthma. *J Allergy Clin Immunol* 2004;113:610–619.
58. Ricciardolo FL, Sterk PJ, Gaston B, Folkerts G. Nitric oxide in health and disease of the respiratory system. *Physiol Rev* 2004;84: 731–765.
59. Que LG, Liu L, Yan Y, Whitehead GS, Gavett SH, Schwartz DA, Stamler JS. Protection from experimental asthma by an endogenous bronchodilator. *Science* 2005;308:1618–1621.
60. Gaston B, Sears S, Woods J, Hunt J, Ponaman M, McMahon T, Stamler JS. Bronchodilator *S*-nitrosothiol deficiency in asthmatic respiratory failure. *Lancet* 1998;351:1317–1319.
61. Wu H, Romieu I, Sienra-Monge JJ, Estela Del Rio-Navarro B, Anderson DM, Jenchura CA, Li H, Ramirez-Aguilar M, Del Carmen Lara-Sanchez I, London SJ. Genetic variation in *S*-nitrosoglutathione reductase (GSNOR) and childhood asthma. *J Allergy Clin Immunol* 2007;120:322–328.
62. Kiley J, Smith R, Noel P. Asthma phenotypes. *Curr Opin Pulm Med* 2007;13:19–23.
63. Reddel HK, Taylor DR, Bateman ED, Boulet LP, Boushey HA, Busse WW, Casale TB, Chanez P, Enright PL, Gibson PG, *et al.* An official American Thoracic Society/European Respiratory Society statement: asthma control and exacerbations: standardizing endpoints for clinical asthma trials and clinical practice. *Am J Respir Crit Care Med* 2009; 180:59–99.