Use of exhaled nitric oxide measurement to identify a reactive, at-risk phenotype among patients with asthma

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Abstract

Background: Exhaled nitric oxide (FE_{NO}) is a biomarker of airway inflammation in mild to moderate asthma. However, whether FE_{NO} levels are informative regarding airway inflammation in severe asthma patients, who are refractory to conventional treatment, is unknown. Here, we hypothesized that classification of severe asthma based on airway inflammation as defined by FE_{NO} levels would identify a more reactive, at risk asthma phenotype. **Methods:** FE_{NO} and major features of asthma, including airway inflammation, airflow limitation, hyperinflation, hyperresponsiveness and atopy, were determined in 446 individuals with different degrees of asthma severity (175 severe, 271 non-severe) and 49 healthy subjects enrolled in the Severe Asthma Research Program. **Results:** FE_{NO} levels were similar among severe and non-severe asthma patients. The proportion of individuals with high FE_{NO} levels (> 35 ppb) was the same (40%) among groups despite greater corticosteroid therapy in severe asthma. All asthmatics with high FE_{NO} had more airway reactivity (maximum reversal in response to bronchodilator administration and by methacholine challenge), more evidence of allergic airway inflammation (sputum eosinophils), more evidence for atopy (positive skin tests, higher serum IgE and blood eosinophils), and more hyperinflation, but decreased awareness of their symptoms. High FE_{NO} identified those severe asthma patients characterized by the greatest airflow obstruction and hyperinflation and most frequent utilization of emergency care. **Conclusion:** Grouping of asthma by FE_{NO} provides an independent classification of asthma severity; and among severe asthmatics identifies the most reactive and worrisome asthma phenotype.

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Introduction

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. While 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 non-invasive, easy to perform test to monitor severe asthmatics 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 (FE_{NO}) reflects the redox state of the airway and has been proposed as a marker of airway inflammation and guide for anti-inflammatory therapy in asthma (7). High levels of FE_{NO} are well documented in non-severe asthma (8-21) and decrease in response to treatment with corticosteroids (22-27). However, measures of FE_{NO} in 50 severe asthma patients in the European multicenter study of chronic severe asthma suggests that FE_{NO} levels of severe asthma patients, who are refractory to conventional treatments, may not be suppressed by corticosteroids (28). Although the mean FE_{NO} levels of severe asthmatics were similar to non-severe asthmatics, 22 (44%) of the severe asthmatic subjects who were receiving high dose oral corticosteroids had three-fold higher FE_{NO} than those receiving inhaled corticosteroids, which suggested a substantial subpopulation of severe asthmatics 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 FE_{NO} levels would identify a more severe asthma phenotype. The present study was designed to assess alterations of FE_{NO} in severe asthmatics as compared to non-severe asthmatics and healthy controls, and the relationship between FE_{NO} and asthma severity, airflow limitation, hyperinflation, hyper responsiveness and atopy. While the average FE_{NO} levels in severe and non-severe asthma were previously reported to be similar (29), when asthma was classified based on FE_{NO} levels, a distinct asthma phenotype emerged. In general, asthmatics with high FE_{NO} 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, severe asthmatics with high FE_{NO} levels had the greatest airway reactivity, most hyperinflation and the least awareness of their asthma symptoms. The findings provide evidence that FE_{NO} levels are informative for classification of severe asthma phenotypes and allow identification of a particularly worrisome subgroup of severe asthmatics. Some of the results of these studies have been previously reported in the form of an abstract (Reference).

Materials and Methods

Detailed methods and statistical analyses are provided in an 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 2 puffs of inhaled albuterol), methacholine provocation, and allergy prick skin testing to a standard panel of aeroallergens. Subjects were non-smokers, and classified as healthy controls 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 by 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 cc either spontaneously or after 2 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 on an automated spirometer consistent with American Thoracic Society standards (30). Plethysmographic lung volumes, including total lung capacity (TLC) and residual volume (RV) were measured in 62 Severe and 53 Non-severe Asthma subjects using methods conforming to ATS guidelines (31), and recorded as the percent of predicted values

(%Prd) obtained with the equations of Stocks and Quanjer (32), with adjustments for African Americans per ATS recommendations (33).

Atopy

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

Exhaled NO (FE_{NO})

All SARP centers performed on-line and/or off-line NO measurements according to the standards published by the American Thoracic Society (ATS)(34). Online FE_{NO} values were used in all data analyses in this report. NO levels were measured online by chemiluminescence at a constant expiratory flow (50ml/sec) in all participating centers. The analyzers were calibrated in accordance with the manufacturer's instructions. Because spirometry can affect the FE_{NO} levels, exhaled gases were collected prior to spirometry, if completed on the same day. Based on recent data suggesting poor asthma control when FE_{NO} was more than 35 ppb (7), we evaluated clinical characteristics of asthma populations in subgroups of high (>35ppb) and low (<35ppb) 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, this figure provided the rationale for selecting 35 ppb as a cutoff point for high and low FE_{NO} that is the basis for all data analyses in this study. Relevant variables (as outlined in the table provided in the supplement) in the database were analyzed using receiver operator characteristics (ROC) curves with FE_{NO} as a continuous variable. The cutoff point for each variable was determined based on these ROC curves. This figure represents the frequency

distribution of all these cut off points. The median of all cutoff points for the variables (both categorical and continuous) that showed a significant relationship with FE_{NO} was 37 ppb. This provided support for the validity of our selection of 35 ppb as the cutoff point between high and low FE_{NO}.

Total NO reaction products (NOx)

NO reaction products 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) (35).

Statistical analyses

Categorical data were summarized by frequencies, and statistical comparisons for categorical variables 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 the sample size, mean and standard deviation (SD), and alternatively using the median and interquartile range (IQR) for variables with skewed distributions. Associations between NO levels and other variables were assessed using linear regression for FE_{NO} as a continuous variable and multiple logistic regression for FE_{NO} (high or low) as categorical variables. Multiple logistic regression modeling will be described in more detail in the Results section. All tests and model fitting were performed with the JMP statistical program Version 5.0 (SAS Institute Inc, Gary, NC, USA) and R version 2.4.1 (www.R-project.org) (36). Models for FE_{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 in order to avoid confounding that would render the estimated associations with the outcome as non-interpretable or misleading. Similarly, a logistic regression model for which the FE_{NO} outcome would be classified as high or low had to be parsimonious in order to be mathematically stable.

Results

Characterization of study population

On-line FE_{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 controls and non-severe asthmatics were younger than severe asthmatics ($p<0.05$) (Table 1). As expected, lung functions were lower in severe asthmatics than in non-severe or healthy controls (Table 1). The detailed clinical description of individuals in the SARP dataset 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 (37).

NO in asthma

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

The high NO phenotype in asthma

There were equal proportions of severe and non-severe asthmatics in the low and high FE_{NO} groups. In general, asthmatics with high FE_{NO} demonstrated several distinct characteristics when compared to asthmatics with low FE_{NO}. Demographically, asthmatics with high FE_{NO} were younger [Age in yrs (Mean ± SD): low FE_{NO} 38 ± 12, high FE_{NO} 36 ± 13; p= 0.03] and diagnosed with asthma at a younger age [Age in yrs (Mean ± SD): FE_{NO} 16 ± 13, high FE_{NO} 14 ± 14; p=

0.05] and less likely to be female [Female (%) of population, low FE_{NO} 70%, high FE_{NO} 60%; p= 0.02].

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

High FE_{NO} asthmatics whether severe or non-severe were more likely to be atopic as shown by more positive skin tests [number of positive skin tests (Mean ± SD): low FE_{NO} 3.4 ± 3, high FE_{NO} 4.2 ± 3; p= 0.004], higher serum IgE level [Serum IgE (Mean ± SD): low FE_{NO} 219 ± 366, high FE_{NO} 340 ± 402; p= 0.0001], and higher blood eosinophils [% blood eosinophils (Mean ± SD): low FE_{NO} 3.4 ± 3.7, high FE_{NO} 5.1 ± 3.9; p= 0.0001]. They also had more eosinophils in the sputum [% sputum eosinophils (Mean ± SD): low FE_{NO} 3 ± 7, high FE_{NO} 13 ± 23; p= 0.0001] suggesting more evidence of allergic airway inflammation. Interestingly, asthmatics with high FE_{NO} levels were less likely to have seen a physician in the last 12 months [%: low FE_{NO} 72%, high FE_{NO} 63%; p= 0.04], but more likely to have been in the emergency room [%: low FE_{NO} 66%, high FE_{NO} 73%; p= 0.05] over the same time period, or admitted to the intensive care unit in the past [%: low FE_{NO} 16%, high FE_{NO} 25%; p= 0.02].

Asthmatics with low NO levels were more likely to be overweight [BMI in kg/m^2 (Mean±SD: low FE_{NO} 31±9, high FE_{NO} 28±8; p= 0.002], have systemic hypertension [%: low FE_{NO} 16%, high FE_{NO} 8%; p= 0.05], and be on treatment for diabetes [%: low FE_{NO} 40%, high FE_{NO} 11%; p= 0.01].

Characterizing the high FE_{NO} *phenotype in severe asthma*

In patients with severe asthma, high FE_{NO} levels identified a phenotype that appeared to be the most severe of all groups, including low- FE_{NO} severe, or high or low- FE_{NO} non-severe groups. Severe asthmatic individuals with high FE_{NO} levels tended to share several characteristics. They had the greatest airway reactivity of any group defined by 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 FE_{NO}, NO metabolites (NOx) in serum were higher in all severe asthmatics as a group in comparison to non-severe asthmatics [NOx uM: non-severe 36 ± 23, severe 42 ± 24; p= 0.0009] and were unrelated to FE_{NO} 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 FE_{NO} *phenotype in non-severe asthma*

In patients with non-severe asthma, high FE_{NO} similarly identified a more severe subgroup. In fact, the non-severe patients with high FE_{NO} were shared more similarities with severe asthmatics with high FE_{NO} than with non-severe asthmatics with low FE_{NO}. For instance, the non-severe group with high FE_{NO} 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 non-severe asthmatics with low FE_{NO} levels. They also had eosinophilic inflammation (Table 4) and more intensive care unit (although not emergency room) admissions. These individuals were the thinnest among all groups (Table 3).

FE_{NO} and lung volumes

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

FE_{NO} and use of corticosteroids and other medications

The greater reactivity in the high FE_{NO} asthma subgroups suggested that these patients had greater airway inflammation and/or less anti-inflammatory 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 asthma patients with high or low FE_{NO}, but more patients in the high FE_{NO} group were on oral corticosteroids (Table 5). Individuals with high FE_{NO} were more likely to be on theophylline (Table 5). When the corticosteroid use was further analyzed by severity in addition to FE_{NO} levels, again there was no significant difference in inhaled corticosteroid use between the high or low FE_{NO} groups regardless of severity. The high FE_{NO} severe asthma group had the highest proportion of oral corticosteroid use [%oral corticosteroid use: Severe-low FE_{NO} 37%, Non-severe-low FE_{NO} 1%, Severe-high FE_{NO} 56%, Non-severe high FE_{NO} 5%; p= 0.01]. While only a small number of individuals were taking theophylline, severe asthmatics with high FE_{NO} levels were much more likely to be on daily theophylline than any of the other groups [%Theophylline use: Severe-low

FE_{NO} 13%, Non-severe-low FE_{NO} 1%, Severe-high FE_{NO} 29%, Non-severe high FE_{NO} 3%; p= 0.01]. Thus, the finding of high FE_{NO} in the severe or non-severe asthma subgroups was likely not due to less corticosteroid therapy than the low FE_{NO} subgroups. Multivariate analyses and modeling for determinants of FE_{NO} did not indicate an influence of corticosteroid use on FE_{NO} levels (Table 6).

Discussion

This study provides evidence that subclassification by FE_{NO} defines asthma phenotypes independent of current definitions for asthma severity. Asthmatics who have high FE_{NO} levels share several characteristics regardless of their asthma severity as it is currently defined. Asthmatics with high FE_{NO} 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 FE_{NO} 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 FE_{NO} identifies a severe asthma phenotype that has the greatest eosinophilic airway inflammation, the most severe airflow limitation, and utilizes 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 (38, 39). Abnormalities in NOS1 and 2 genotype and expression are associated with asthma and increased NOS activity is associated with increased FE_{NO} levels (40-42). FE_{NO} 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 (43).

The independent association of elevated FE_{NO} 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 (44-46). Further studies are needed to determine the nature of the interaction between NO and TLC in asthma, but the current study shows that FE_{NO} is associated with hyperinflation in asthma. Furthermore, FE_{NO} in severe asthma might reflect airway remodeling processes (47, 48). Since many of the variables that are related to NO are also related to severity, relationships between variables and FE_{NO} 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 FE_{NO} and markers of atopy and eosinophilic inflammation was confirmed in non-severe asthma, which suggests a strong dependence of FE_{NO} on these variables. However, the multivariate significance of factors that influence FE_{NO} in all asthma was driven primarily by the non-severe asthma group. Most features did not significantly influence FE_{NO} in severe asthma. This suggests that features other than the ones evaluated in this study may be determinants of high FE_{NO} levels in severe asthma.

There are several possible explanations for the presence of high FE_{NO} in severe asthma patients. Since FE_{NO} levels of non-severe asthmatics 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 FE_{NO}. In this context, one possible explanation for the high FENO may be inadequate corticosteroid therapy. However, high FENO levels in severe asthmatics 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 severe asthmatics with high FENO are less compliant than severe asthmatics with low FENO, even though they report similar corticosteroid use. Importantly, high FENO 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 non-severe asthma. This may partially explain why the patients with high FE_{NO} have similar characteristics in both patient groups." Taken together with previous studies (1, 3, 23, 28, 49-53) , the high FE_{NO} severe asthma group may have relative resistance to corticosteroid therapy. In support of this, severe asthmatic individuals with high FE_{NO} are more likely to be on 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 FE_{NO} group is another indirect marker of more difficult to treat, less-corticosteroid responsive asthma.

Analyses of the characteristics of low FE_{NO} severe asthma in this study also provide new information on asthma phenotypes. FE_{NO} levels reflect a balance between the rates of NO production and its consumption, which is largely related to oxidant-NO reactions (10, 54, 55). Thus, low levels of FE_{NO} in asthma may be related to less NO synthesis or greater oxidative consumption. Mechanisms that affect NO production include factors that modify NOS enzyme activity or expression, alter non-enzymatic release of NO from storage pools, or change the denitrifying organisms that colonize the upper airway (56-60). However, the end products of NO consumption are greater in severe asthma than in non-severe, which suggests that total NO production is greater in severe asthma but may not be reflected by levels in the exhaled breath due to 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 FE_{NO} asthma group, i.e. higher BMI, 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 cohort and not a randomized controlled trial. FENO levels were analyzed in a

cross sectional fashion and not based on or before or after an intervention. Certain variables like compliance with therapy could not be completely accounted for and verified.

Clinical asthma phenotypes have been recognized for some time (50, 61), but quantitative biomarkers have not been previously identified in severe asthma (50). 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 (50). While the current definition of asthma severity is very useful for clinical research, it is cumbersome to use and impractical for the busy office setting. The availability of an easy to measure, non-invasive marker would greatly simplify and improve severe asthma management (62). 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, hyper-reactivity, hyperinflation, and persistent airway inflammation. While the retrospective nature of our analysis has precluded us from determining whether FENO could predict future risk of exacerbations in asthma, its correlation with ER visits and hospital and ICU admission suggests a great potential for FENO 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 severe asthmatics, who are refractory to therapy.

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Table 1: Demographics, Pulmonary function, exhaled nitric oxide levels, and Corticosteroids usage for all subjects.

	Controls	N	Non-Severe	N	Severe	N
Total subjects in each group	49		271		175	
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
FE_{NO} (ppb)	17 ± 9	49	43 ± 42	271	42 ± 41	175
Median (IQ range) *	14 (11-19)	49	27 (17-55)	271	27 (17-52)	175
Gender (male)	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 (IQ range) *	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 (IQ range) *	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 (IQ range) *	2 (1-2.85)	45	3.7 (2-5)	252	3 (1.5-5)	168

N: number of individuals with available data

Race C, Caucasian; AA, African American; AI: American Indian or Alaska native; A, Asian; NH, Native Hawaiian; O: other, U: uncertain; R: refused; MR, multiple races

* indicates Fisher's, ANOVA, or Kruskal-Wallis $p<0.05$ among 3 groups.

Table 2: Pulmonary function by FE_{NO}.

Characteristic	Low FE_{NO} (≤ 35ppb)	N	High FE_{NO} (> 35 ppb)	N	p
Baseline FVC % predicted	85±18	267	87±19	179	0.20
Maximum FVC % of predicted	93±16	253	100±15	170	<0.001
Baseline FEV1 % predicted	74±20	267	73±23	179	0.80
Maximum FEV1 % of predicted	85±20	253	90±18	170	0.005
FEV1/FVC ratio % of predicted	86±14	267	81±14	179	<0.001
Maximum FEV1 reversal %	14±16	253	20±17	170	
Median (IQ range) *	10 (5-18)	253	16 (8-26)	170	<0.001
PC20	4.3±6	203	1.7±3	123	
Median (IQ range) *	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

* Wilcoxon rank sum P-values reported rather than T-test.

Table 3: Pulmonary function and FE_{NO} level by severity.

Characteristic	Severe low FE_{NO}	N	Non-severe low FE_{NO}	N	Severe high FE_{NO}	N	Non-severe high FE_{NO}	N	Low FE_{NO}: Severe vs. non-Severe	High FE_{NO}: Severe vs. non-Severe	Severe: Low vs. High FE_{NO}	Non-severe: Low vs. High FE_{NO}
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
FEV1/FVC ratio % predicted	79±15	105	90±11	162	74±14	70	86±12	109	<0.001	<0.001	0.011	0.03
Maximum FEV1 reversal %	18±23	101	11±9	153	23±19	67	17±15	103				
Median (IQ range) *	14 (6-22)	101	8 (5-14)	153	21 (9-29)	67	13 (7-22)	103	0.002	0.01	0.005	<0.001
PC20	3.9±6	53	4.4 ±6	149	1.5±3	27	1.7±3	96				
Median (IQ range) *	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 months	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

High FE_{NO} defined as > 35 ppb, and low FE_{NO} by $\leq$ 35 ppb

* Wilcoxon rank sum P-values reported rather than those based on contrasts from ANOVA.

Table 4: Inflammatory cells in the blood, BAL and sputum by severity and FE_{NO} levels.

Characteristic	Severe low FE_{NO}	N	Non-severe low FE_{NO}	N	Severe high FE_{NO}	N	Non-severe high FE_{NO}	N	Low FE_{NO}: Severe vs. non-Severe	High FE_{NO}: Severe vs. non-Severe	Severe: Low vs. High FE_{NO}	Non-severe: Low vs. High FE_{NO}
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 WBC (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 WBC (%)	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

Table 5: Medication use by FE_{NO} levels.

Characteristic	Low FE_{NO} (≤ 35ppb)	N	High FE_{NO} (> 35 ppb)	N	P*
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 beta agonist	57%	267	50%	179	0.1
Total beta agonists	91%	267	91%	179	0.9
Total long acting beta 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

Table 6: Results of multivariate logistic regression analysis with high FE_{NO} (>35 ppb) as the outcome.

Characteristic	All asthma n=335 Odds ratio (95% CI)	All asthma P value	Non-severe asthma n=210 Odds ratio (95% CI)	Non-severe asthma p-value	Severe asthma n=125 Odds ratio (95% CI)	Severe asthma 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 Maximum FEV1 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 months	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 months	2.33 (1.20 - 4.69)	0.01	2.65 (0.87 - 7.90)	0.09	2.5 (1.08 - 7.69)	0.05

The number "n=" reflects the number of individuals who had a complete set of all variables to run the model.
BMI: body mass index, CS: corticosteroids, ICS: inhaled CS, ER: emergency room

Figure Legend: Relevant variables (as outlined in the table provided in the supplement) in the database were analyzed using receiver operator characteristics (ROC) curves with FENO as a continuous variable. The cutoff point for each variable was determined based on these ROC curves. This figure represents the frequency distribution of all these cut off points. The median of all cutoff points for the variables (both categorical and continuous) that showed a significant relationship with FENO was 37 ppb. This provided support for the validity of our selection of 35 ppb as the cutoff point between high and low FENO.

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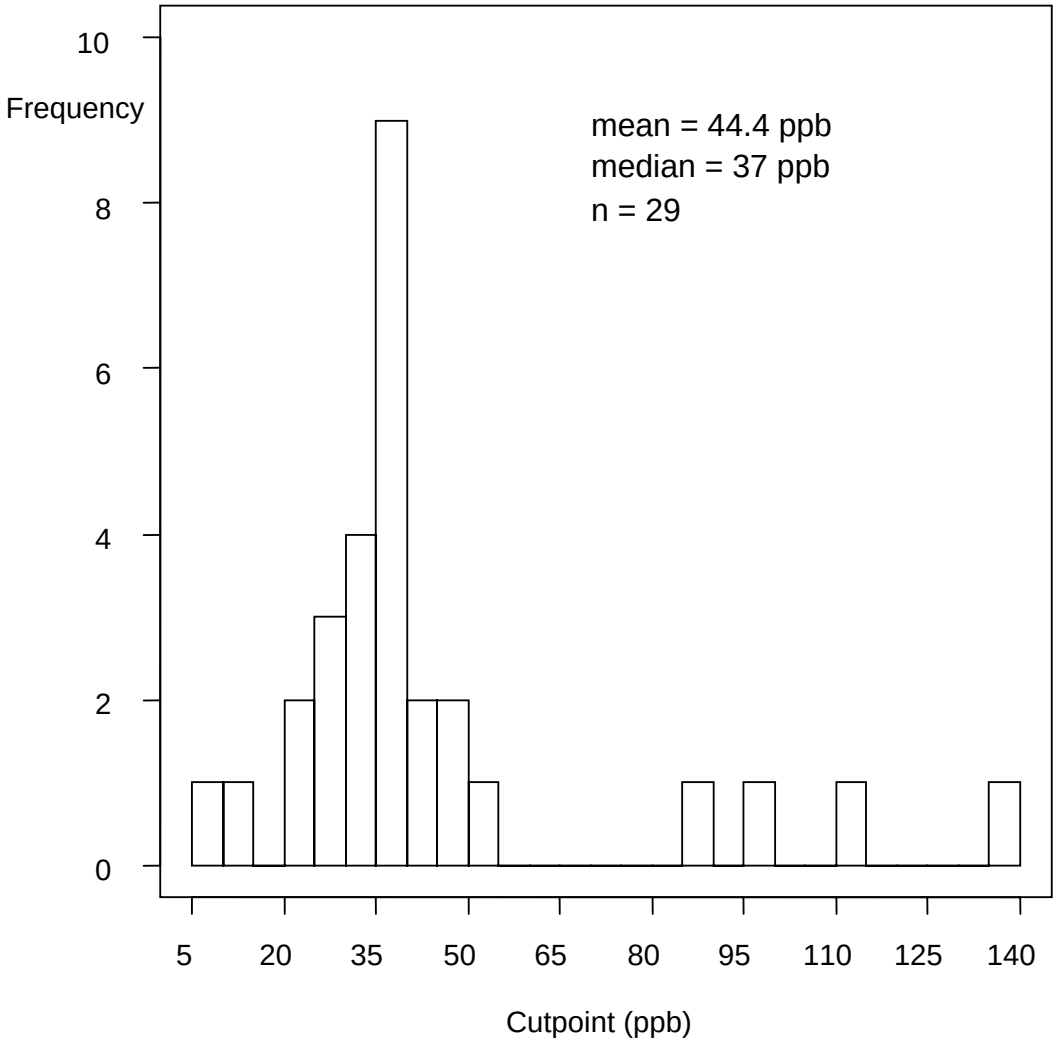
Figure


10
Frequency
8
6
4
2
0
mean = 44.4 ppb
median = 37 ppb
n = 29
5
20
35
50
65
80
95
110
125
140
Cutpoint (ppb)