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ORIGINAL ARTICLE

Predictors for Identifying the Efficacy of Systemic Steroids on Sustained Exhaled Nitric Oxide Elevation in Severe Asthma

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ABSTRACT

Background: Some patients with asthma have high levels of exhaled nitric oxide fraction (FE_{NO}) despite inhaled corticosteroids (ICS) therapy. Early studies suggested that this might be explained by the presence of heterogeneous airway inflammation. We aimed to assess the predictors for identifying the efficacy of systemic corticosteroids on residual FE_{NO} elevations in severe asthma.

Methods: Twenty severe asthmatics with persistent FE_{NO} elevation (≥40 ppb) despite maintenance therapy including high-daily-dose ICS were enrolled. Asthma Control Questionnaire (ACQ), lung function, blood eosinophils, and FE_{NO} were assessed before and after 14 days treatment with 0.5 mg/kg oral prednisolone/day.

Results: ACQ, blood eosinophils, FE_{NO} level, FVC, FEV_1, FEV_1/FVC ratio and the slope of the single nitrogen washout curve (ΔN_2) were significantly improved by treatment with prednisolone. 70% of the subjects showed ≥20% reductions in the FE_{NO} levels. The reduction in FE_{NO} levels was significantly correlated with the improvements in ACQ ($p < 0.0001$), FVC ($p < 0.01$), FEV_1 ($p < 0.0001$), and ΔN_2 ($p < 0.05$). Among the measurements at baseline, the FE_{NO} levels and blood eosinophil numbers were identified as significant predictors of ≥20% reductions in the FE_{NO} levels by systemic steroid therapy.

Conclusions: Systemic corticosteroids could suppress the residual FE_{NO} elevations in more than half of the patients with severe asthma and the reduction in FE_{NO} levels was associated with improvements in asthma control and airflow limitation. The FE_{NO} levels and blood eosinophil numbers were the predictors of improved residual airway inflammation by systemic steroid therapy in severe asthma.

KEY WORDS

airflow limitation, airway inflammation, eosinophils, inhaled corticosteroid

ABBREVIATIONS

ACQ, Asthma Control Questionnaire; DL_{CO}, Diffusion capacity of the lung for carbon monoxide; dN_2, Third phase slope of the single nitrogen washout curve; FE_{NO}, Exhaled nitric oxide fraction.

INTRODUCTION

Asthma is an inflammatory disorder of the airways, and inhaled corticosteroids (ICS) are widely used for the long-term management of the disease.[1] The exhaled nitric oxide fraction (FE_{NO}) is a useful surrogate marker to assess airway inflammation.[2] The FE_{NO} levels are elevated in steroid-naive asthma[3] and are generally reduced in a dose-dependent manner by treatment with ICS.[4] However, not all asthma patients respond to corticosteroids similarly and there is considerable variation in the FE_{NO} levels.[5] Indeed, the FE_{NO} remains persistently high despite ICS therapy in some individuals.[6-10]

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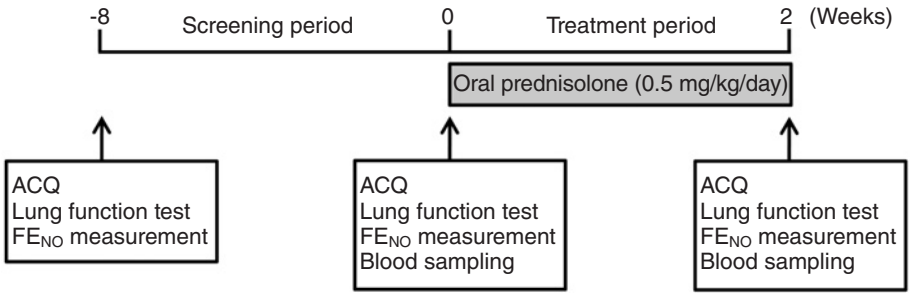

Fig. 1 Study design.

A common cause of persistently high levels of FE_{NO} is poor adherence to ICS therapy. Other explanations could be poor inhaler technique, inadequate dose of ICS, or persistent exposure to allergen.[2] Sustained high FE_{NO} may occur even in subjects without respiratory symptoms.[2] However, recent studies have shown that the grouping of asthma by FE_{NO} provides an independent classification of asthma severity, and the subgroups with sustained high FE_{NO} tend to be a highly reactive asthma phenotype.[9,10] There is also evidence that airway hyperresponsiveness as well as high levels of FE_{NO} are related to exacerbations of asthma and accelerated decline in lung function.[11-14] Moreover, excessive nitric oxide (NO) synthesis was found in individuals with refractory asthma.[15] These data might be explained by the presence of heterogeneous airway inflammation that is resistant to ICS therapy.[10,15-18] Among patients with refractory asthma, it is speculated that some proportion of individuals are truly steroid resistant, which is defined as no clinical improvement after treatment with systemic corticosteroids.[18] However, the predictors that can stratify patients who can be expected to show improved persistently high levels of FE_{NO} by treatment with systemic steroids have not been fully elucidated in severe asthma.

In the present study, severe asthma patients with persistently high levels of FE_{NO} (≥40 ppb) despite maintenance therapy including high-daily-dose ICS were enrolled. We assessed the response to high-dose systemic corticosteroids in terms of asthma control, lung function, blood eosinophils, and FE_{NO} level. The potential of objective measurements made at baseline for predicting the efficacy of systemic steroids on residual airway inflammation was analyzed.

METHODS

STUDY SUBJECTS

Subjects over 20 years old were considered eligible if they satisfied the standard criteria for asthma.[19] All subjects were recruited from June 2012 to August 2012. The criterion for refractory asthma from the American Thoracic Society was used to determine the severity classifications.[20] The definition of severe asthma required a combination of 1 of 2 major (oral steroids for ≥50% of year, high-dose ICS) and 2 of 7 minor criteria (daily second controller medication, near-daily basis short-acting β2-agonist use, persistent airflow limitation, ≥1 urgent care visit/year, ≥3 oral steroids burst/year, deterioration with reduced steroids, near-fetal event in the past). Subjects were excluded if they were current smokers, had had an exacerbation of asthma or had been treated with systemic steroids 8 weeks prior to the study. Also, patients with poor adherence to the treatment (defined <80% adherence based on prescription refill data) or with other pulmonary diseases were excluded. Complete blood cell count, differential count of leukocytes, total immunoglobulin E (IgE), and specific IgE for common inhaled allergens were examined. Positive specific IgE to at least one allergen was assumed to confirm the presence of atopy. All ex-smokers had chest computed tomography to exclude clinically occult emphysema. This trial was approved by the local

Table 1 Demographics of study subjects at baseline

Number (male/female)	20 (10/10)
Age (years)	53.5 ± 12.1
Body mass index (kg/mm^2)	22.8 ± 3.9
Smoking status (never/ex)	9/11
Smoking index (pack-years)	16.6 ± 3.6
Atopy, *n* (%)	18 (90.0)
Serum total immunoglobulin E (IU/mL)	780 ± 951
Allergic rhinitis, *n* (%)	17 (85.0)
Chronic rhinosinusitis, *n* (%)	6 (30.0)
FVC (L)	3.16 ± 0.98
FVC % of predicted (%)	87.7 ± 9.8
FEV_1 (L)	2.01 ± 0.76
FEV_1/FVC ratio (%)	63.2 ± 13.9
FEV_1 % of predicted (%)	66.2 ± 13.5
Inhaled corticosteroids, *n* (%)	20 (100.0)
Dose of inhaled corticosteroids (μg/day) †	1760 ± 228
Inhaled long-acting β_2-agonist, *n* (%)	20 (100.0)
Leukotriene receptor antagonist, *n* (%)	13 (65.0)
Theophylline, *n* (%)	11 (55.0)

Values are Means ± SD. †Inhaled corticosteroids, expressed as beclomethasone dipropionate equivalent.

Table 2 Changes in lung function, asthma control, blood eosinophils, and FE_{NO} levels during the study period

Characteristics	Screening period (-8 weeks)	Pre-steroid treatment (Baseline)	Post-steroid treatment (2 weeks)	*p* value -8 weeks vs. baseline	*p* value baseline vs. 2 weeks
FVC (L)	3.18 ± 0.98	3.16 ± 0.98	3.36 ± 0.89	0.42	<0.01
FEV_1 (L)	1.99 ± 0.81	2.01 ± 0.76	2.25 ± 0.73	0.34	<0.01
FEV_1/FVC ratio (%)	62.2 ± 14.7	63.2 ± 13.9	66.8 ± 13.8	0.31	<0.01
ΔN_2 (%)	-	2.2 ± 1.1	1.4 ± 0.7	-	<0.01
DL_{CO}/V_A (%)	-	105.4 ± 15.7	103.3 ± 12.7	-	0.44
ACQ (points)	1.9 ± 0.7	1.8 ± 0.6	1.1 ± 0.6	0.54	<0.01
Blood eos (cells/μL)	593 ± 278	561 ± 310	153 ± 158	0.61	<0.01
FE_{NO} (ppb)	63.2 ± 19.4	60.1 ± 21.3	38.0 ± 16.3	0.23	<0.01

Abbreviations: ΔN_2, the third phase slope of the single nitrogen washout curve; DL_{CO}, diffusion lung carbon monoxide; V_A, alveolar volume; eos, eosinophils; ACQ, Asthma Control Questionnaire; FE_{NO}, exhaled nitric oxide fraction. Values are Means ± SD. Wilcoxon signed rank test *p* values are reported to compare the groups.

ethics committee (IRB #526) and registered with the University hospital Medical Information Network (UMIN 000008401). Informed written consent was obtained from each participant.

STUDY DESIGN

This prospective observational study evaluated the response to oral prednisolone in terms of asthma control, lung function, blood eosinophils, and airway inflammation (Fig. 1). During the screening period, FE_{NO} was measured before and after 8 weeks observation, and severe asthmatic patients with sustained high levels of FE_{NO} (≥40 ppb) were enrolled. We employed Asthma Control Questionnaire (ACQ), lung function test, blood sampling, and FE_{NO} measurement before and after 14 days treatment with 0.5 mg/kg oral prednisolone · day^{-1}. Asthma maintenance treatment was continued without any changes during the study period.

ASTHMA CONTROL QUESTIONNAIRE (ACQ), LUNG FUNCTION, AND EXHALED NO

FE_{NO} was measured by a chemiluminescence analyzer (modified NA-623N®; Chest, Tokyo, Japan) as previously described.[21] It has been confirmed that the FE_{NO} values measured by this device is in agreement with those of a widely used electrochemical analyzer (NIOX MINO; Aerocrine, Solna, Sweden).[22] Based on our study,[10] we selected 40 ppb as the cutoff point for high and low FE_{NO}, a value that was within previously published cutoff points ranging from 35 to 50 ppb.[2,9,23] The forced vital capacity (FVC), forced expiratory volume in 1 s (FEV_1), the third phase slope of the single nitrogen washout curve (ΔN_2), and diffusion lung carbon monoxide (DL_{CO}) were measured using a dry rolling seal spirometer (CHESTAC-8800; Chest).[21] The changes in each variable were estimated according to the following criteria: difference in ACQ score; percentage changes in FVC, FEV_1, ΔN_2, and FE_{NO} (post-steroid level - pre-steroid level/pre-steroid level × 100). Steroid responsiveness of airway inflammation was defined as ≥20% reduction in the FE_{NO} levels.[2] The ACQ-5 is a questionnaire that assesses the asthma control according to five items, each of which can be rated on a seven point scale.[24] The overall score was the mean of the five responses. This questionnaire has been used in earlier studies in Japan.[25]

STATISTICAL ANALYSIS

All data were expressed as mean values ± standard deviation. The measurements at different time points were compared by Wilcoxon signed rank test. Spearman's correlation analysis was performed to assess the correlation. Receiver operating characteristic curves were plotted to estimate the predictive value of the baseline measurements for assessing the response to oral steroids of airway inflammation. A *p*-value of <0.05 was considered significant.

RESULTS

The baseline characteristics are listed in Table 1, 2. A total of 20 patients with severe asthma completed the study. Despite treatment with high-daily-dose ICS (mean equivalent dose of 1,760 μg beclomethasone dipropionate/day), all study subjects were using second controller medications, and the mean ACQ score was 1.8. These characteristics were consistent with the criteria for severe asthma. In addition, there was no significant difference in ACQ score, lung function, and the FE_{NO} levels between the screening period (-8 weeks) and the beginning of the treatment period (baseline) (Table 2).

As shown in Table 2, oral steroids significantly reduced the FE_{NO} level from 60.1 ppb at baseline to 38.0 ppb at day 14 ($p < 0.01$). 14 of the subjects (70%) showed ≥20% reduction in FE_{NO} levels (60% of the subjects showed reductions in FE_{NO} of <40 ppb). Significant improvements in ACQ score, FVC, FEV_1, FEV_1/FVC, ratio, ΔN_2, and blood eosinophil numbers

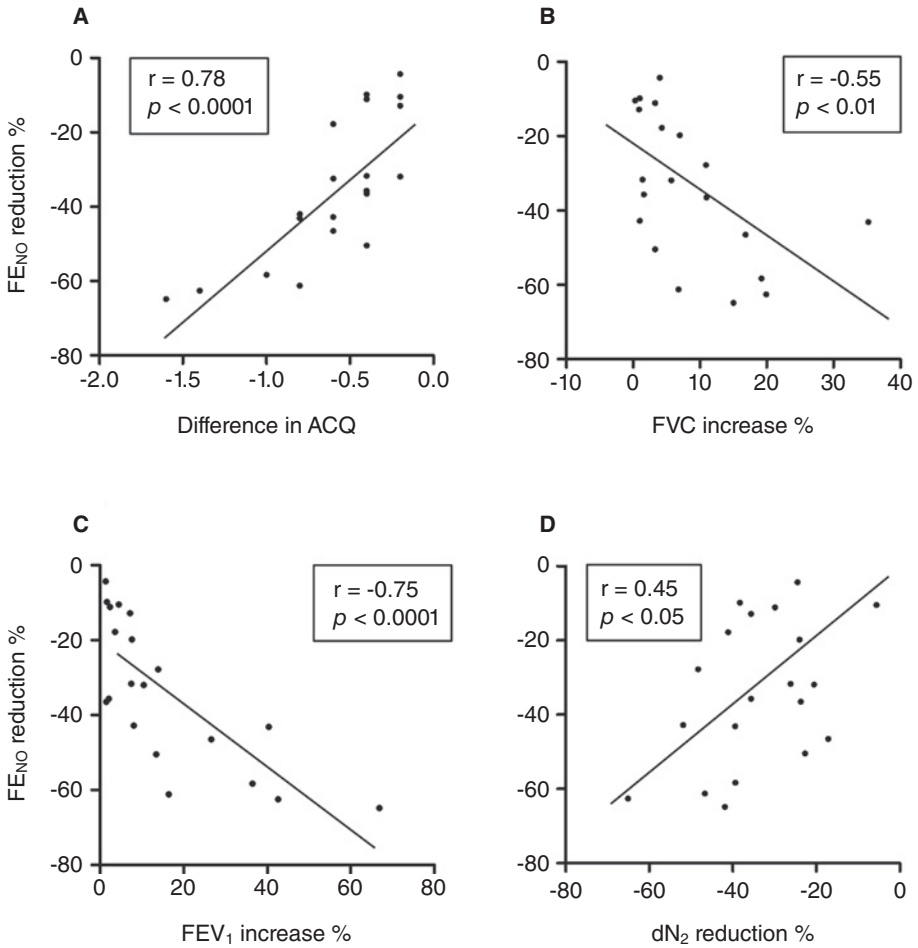

Fig. 2 Relationship between changes in exhaled nitric oxide fraction (FE_{NO}) and (**A**) difference in ACQ score, (**B**) percentage increase in FVC, (**C**) percentage increase in FEV_1, (**D**) percentage reduction in third phase slope of the single nitrogen washout curve (dN_2): The line corresponds to the fitted regression equation. The r value is Spearman's correlation coefficient.

Table 3 Receiver operator characteristic analysis for assessing the predictors of ≥20% reduction in FE_{NO} levels by the treatment with systemic corticosteroids

Predictors	AUC	Cutoff value	Sensitivity	Specificity
ACQ	0.61	1.4	0.50	0.80
FVC % of predicted (%)	0.62	80.3	0.52	0.80
FEV_1 % of predicted (%)	0.57	72.3	0.64	0.60
ΔN_2 (%)	0.65	1.5	0.70	0.64
FE_{NO} (ppb)	0.71	49.7	0.76	0.73
Blood eosinophils (cells/μL)	0.72	416	0.79	0.72

Abbreviations: AUC, area under the curve; ACQ, Asthma Control Questionnaire; ΔN_2, the third phase slope of the single nitrogen washout curve; FE_{NO}, exhaled nitric oxide fraction.

were also found (all $p < 0.01$). Furthermore, the reductions in the FE_{NO} levels were significantly correlated with the improvements in ACQ ($p < 0.0001$), FVC ($p < 0.01$), FEV_1 ($p < 0.0001$), and ΔN_2 ($p < 0.05$) (Fig. 2), and showed a tendency to associate with the reduction in blood eosinophils ($p = 0.08$).

In order to explore whether objective measurements made at baseline might predict the responsiveness to oral steroids of airway inflammation, receiver operating curve analyses were carried out. An area under the curve of >0.7 was considered significant. As shown in Table 3, the FE_{NO} levels and blood eosinophil numbers were identified as predictors of ≥20% reduction in FE_{NO} levels by the treatment with oral steroids (AUC = 0.71, and 0.72, respectively). The values of sensitivity and specificity for selected cutoff values are also listed in Table 3. Indeed, both the blood eosinophil numbers and the FE_{NO} levels at baseline were negatively correlated with the changes in FE_{NO} levels by systemic steroid therapy (r = -0.51, $p < 0.05$, and r = -0.50, $p < 0.05$, respectively) (Fig. 3).

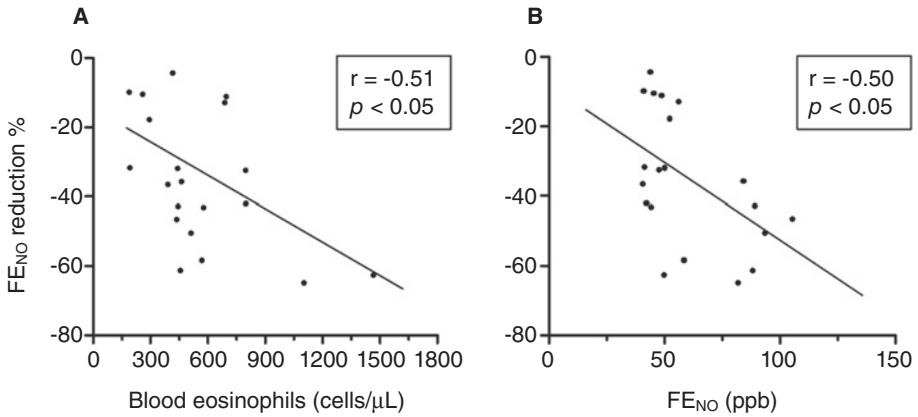

Fig. 3 Relationship between baseline (**A**) blood eosinophil numbers (**B**) exhaled nitric oxide fraction (FE_{NO}) and changes in FE_{NO} levels. The line corresponds to the fitted regression equation. The r value is Spearman's correlation coefficient.

DISCUSSION

In this prospective observational study of severe asthmatics with sustained high levels of FE_{NO}, we found that the ACQ, FVC, FEV_1, FEV_1/FVC ratio, ΔN_2, blood eosinophils, and FE_{NO} were significantly improved by systemic steroid therapy and the reductions in the FE_{NO} levels were related to the improvements in ACQ, FVC, FEV_1, and ΔN_2. The FE_{NO} levels and blood eosinophil counts at baseline were identified as useful predictors of improvement in airway inflammation by treatment with systemic corticosteroids.

High levels of FE_{NO} are known to represent significant airway eosinophilia, and it is also likely to indicate that a symptomatic patient has steroid-responsive airway inflammation.[2] However, in some individuals, the FE_{NO} levels remain persistently high despite adequate anti-inflammatory treatment.[6-10] Recent studies compared asthmatic subjects classified as having high-FE_{NO} with subjects having low-FE_{NO} and identified the common characteristics of this phenotype.[9,10] Asthma patients with high FE_{NO} were atopic and had more eosinophilic airway inflammation, more airway reactivity, more hyperinflation, and the most frequent use of emergency care.[9] Also, the subjects with high-FE_{NO} were characterized by lower FEV_1 values, exaggerated fluctuation of airway caliber, and more severe asthma symptoms.[10] Previous studies suggested that asthma patients with sustained high FE_{NO} may have a vulnerable airway condition that is resistant to corticosteroid.[9,10] Indeed, neutrophilic or persistent eosinophilic inflammation was found in asthmatics with high FE_{NO} despite steroid therapy.[15-18] Interestingly, our study showed that further anti-inflammatory therapy could suppress the residual FE_{NO} elevations in more than half of the patients with severe asthma, and the reduction in the FE_{NO} levels was related to improvements in asthma control and airflow limitation. Our data supports a prior study that showed persistent sputum eosinophilia in patients with severe asthma is not a refractory phenomenon, but is still sensitive to systemic corticosteroids.[17] By contrast, in the present study, 30% of the subjects continued to show high levels of FE_{NO} even after the systemic steroid trial, suggesting that truly steroid resistant inflammatory processes in the airway might also exist among some severe asthmatic patients with sustained high levels of FE_{NO}.[18]

Predicting the responsiveness to anti-inflammatory treatment on residual airway inflammation is important in patients with severe asthma. As expected, the baseline FE_{NO} level was associated with the improvement in sustained airway inflammation by systemic steroids. This may be related to the fact that FE_{NO} is a useful marker of eosinophilic airway inflammation even in patients on ICS therapy,[2] thus show a greater capacity to respond to additive systemic steroid therapy. We speculated that small airways might be involved in the residual airway inflammation because the steroid-mediated reduction in the FE_{NO} levels was correlated with the changes in ΔN_2 values, which reflect airway obstruction in the small airways.[21]

Also, the blood eosinophil number was the useful predictor for identifying the subjects whose sustained high levels of FE_{NO} responded to oral steroids. This finding is novel and interesting given that blood eosinophils play a critical role in pathophysiology of severe asthma. Indeed, an increase in blood eosinophils was found in treated asthmatics and positive correlations were found between the blood eosinophil count and the FE_{NO} levels.[10,16] Also, it has been shown that eosinophils could be one of the sources of the increased NO production[26] and NO production might be involved in facilitating the migration of eosinophils.[27] Taken together, it is suggested that an ongoing recruitment and activation of inflammatory cells may be present in the airways of some severe

asthmatic patients with sustained high FENO even when treated by high-dose ICS. It has been reported that most of the patients with severe asthma on chronic oral steroid therapy required further anti-inflammatory therapy.[28,29] Interestingly, a recent study has shown that a monoclonal antibody against interleukin-5 (mepolizumab) reduces the number of asthma exacerbations in patients with severe eosinophilic asthma, and a multivariate analysis demonstrated that the baseline blood eosinophil count is associated with the efficacy of mepolizumab.[30] Indeed, blood eosinophilia has been identified as an independent risk factor of persistently elevated FENO in treated asthmatics.[10] Taken together, our study suggested that blood eosinophils could be a predictor of the response to systemic anti-inflammatory therapy in severe asthmatic patients with persistent FENO elevations.

There would be some limitations in our study. First, the sample size was relatively small. Second, since the study subjects had severe asthma, airway inflammation was not assessed by cellular examination. Third, we avoided the influence of the cedar pollen season in Japan, but perennial allergen exposure might be a cause of sustained high FENO.[2] Finally, although neither the FENO levels nor lung function showed any difference between the observation period and the beginning of the treatment period, inadequate ICS therapy including poor adherence and/or poor inhaler technique could be an explanation for sustained high FENO in some subjects.

In conclusion, our study showed that systemic steroids could suppress the residual FENO elevations in more than half of the patients with severe asthma, and the reduction in the FENO levels was related to improvements in asthma control and airflow limitation. The FENO levels and blood eosinophil numbers were useful predictors of patients who could be expected to show improved residual airway inflammation by treatment with systemic steroids in severe asthma.

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