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Domiciliary diurnal variation of fractional exhaled nitric oxide for asthma control

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Running head: *FeNO monitoring for asthma control*

Statement: Diurnal variation in FeNO can be used as a biomarker of asthma control and as a predictor of the risk of future exacerbation.

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ABSTRACT

Background:

A major goal of asthma management is maintaining optimal control. Current assessment is based on symptoms and lung function.

Objectives:

We evaluated whether domiciliary daily home FeNO monitoring could be useful as an index of asthma control.

Methods:

Fifty asthmatic subjects and 15 healthy volunteers with a range of asthma severity underwent asthma control questionnaire (ACQ), spirometry before and after salbutamol and sputum induction. FeNO and peak expiratory flow (PEF) were measured twice daily for 2 weeks. A record of exacerbations was obtained 3 months later.

Results:

Diurnal FeNO variation in uncontrolled asthmatics was significantly greater than in controlled asthmatics ($p<0.01$). PEF variation was not different. The daily variation of FeNO levels was also greater in uncontrolled asthmatics compared to controlled asthmatic and healthy subjects ($p<0.01$). 80% of uncontrolled asthmatics experienced at least one or more exacerbations over the 3 months after the enrolment. The combination of diurnal FeNO variation ≥16.6% and ACQ scores ≥ 1.8 was best at predicting uncontrolled asthma (AUC; 0.91, 95%CI: 0.86-0.97, $p<0.001$).

Conclusions:

Diurnal variation in FeNO can be used as a biomarker of asthma control and as a predictor of the risk of future exacerbation. Prospective studies are warranted.

(196 words)

Keywords: Fractional exhaled nitric oxide,daily monitoring, diurnal variation, asthma control, exacerbation, fluctuation

Abbreviations used:

FeNO: Fractional exhaled nitric oxide

ppb: Parts per billion

FEV_1: Forced expiratory volume in one second

FVC: Forced vital capacity

CI: Confidence interval

ATS: American Thoracic Society

ERS: European Respiratory Society

ROC: Receiver operating characteristic

AUC: Area under the curve

ICS; Inhaled corticosteroid

GINA; Global initiative for asthma

ACQ: Asthma control questionnaire

AQLQ: Asthma quality life questionnaire

AHR: Airway hyperresponsiveness

PEF: Peak expiratory flow

LR: Likelihood ratio

PPV: Positive predicted value

NPV: Negative predicted value

BDP: Beclomethasone propionate

ANOVA: Analysis of variance

INTRODUCTION

Over the past decade, there has been a clearer definition of the concepts of asthma severity and control with important implications for the management of asthma. Asthma control is defined by the components of clinical control and future risk of exacerbation, and asthma severity by the requirement for high intensity treatment [1]. The Global Initiative for Asthma (GINA) has also emphasised that a major goal of asthma management is not only achieving and maintaining optimal control but also reducing future risks, particularly those of exacerbations[2]. Symptom questionnaires and spirometry have been recommended for managing asthma[2]. The guidelines have also recommended the need for assessing control over a period of time rather than just at one assessment. Hence, home monitoring can be important for this [3;4] (3;4). Daily home monitoring of peak expiratory flow (PEF) also provides an additional tool for asthma management [5;6].

Using the recommended criteria for determining control, between 20% and 50% of asthma patients have been reported to be uncontrolled [7-9](7-9). Uncontrolled asthma is associated with a decreased quality of life, chronic airflow limitation, a higher risk of having an exacerbation and hospitalisation, associated with a greater probability of death, and an increased economic burden [10-13]. One of the potential reasons for continuing poor level of control is that the current tools available to evaluate control may not be adequate. For example, a marker of inflammation is not currently included in the assessment of control when the level of airway inflammation is generally accepted to contribute to symptoms and to the risk of exacerbations [2].

Measurement of fractional exhaled nitric oxide (FeNO) has been available as an indirect way of assessing the eosinophilic inflammation of asthma [14]. Longitudinal measurements of FeNO in asthmatics may be helpful to predict deterioration [15;16]. These daily fluctuations have been proposed to inform about both severity and control of asthma [17]. With the arrival of portable FeNO monitors, it is now possible for patients to measure FeNO in the home environment on a daily basis [17;18].

We evaluated whether domiciliary diurnal variations and daily

fluctuations of FeNO levels provide useful information to assess asthma control and determine their value for predicting future risks, particularly of asthma exacerbations. In addition, we wished to determine whether these also could provide information about asthma severity. Finally, we determined how comparable this was to the analysis of PEF diurnal measurements.

METHODS

Subjects:

Fifty asthmatic subjects (22 non-severe and 28 severe asthmatics) were recruited from outpatient clinics at the Royal Brompton Hospital and 15 normal healthy volunteers through advertisements. The diagnosis of asthma was made by respiratory physicians according to a clinical history of characteristic symptoms (i.e. dry cough, wheezing, chest tightness and breathlessness) as well as a history of either forced expiratory volume in 1 second (FEV_1) reversibility ≥12% or provocative concentration of methacholine causing a 20% fall in FEV_1 (PC_{20}) of <8mg/ml. Asthmatic subjects were categorized as having controlled or partly controlled, or uncontrolled asthma according to GINA guidelines[2].

Uncontrolled asthmatic subjects were defined as having 3 or more features of the following features over 4 weeks before recruitment: i) daily symptoms more than twice a week, ii) any limitation of activities, iii) any nocturnal symptoms or awakening, iv) need for reliever/rescue treatment more than twice a week, v) spirometry (PEF or FEV_1) <80% predicted or personal best if known[2]. Patients who had less than 2 of these features were assigned as having stable controlled asthma, which includes controlled and partly-controlled asthma. Asthma subjects were also defined as having severe asthma according to the ATS definition of severe therapy-resistant asthma[19].

Subjects were excluded if they were current smokers, had other concomitant respiratory diseases other than asthma, or a respiratory tract infection within 6 weeks of study entry. Healthy volunteers were defined as having a smoking history of less than 5 pack years, no respiratory diseases, and a negative PC_{20} (>16 mg/ml).

All participants gave informed consent to the protocol approved by the Ethics Committee of Royal Brompton & Harefield NHS Trust/National Heart & Lung Institute.

Study design

At study entry, all subjects performed spirometry before and after 4 puffs

of β2-adrenergic agonist (salbutamol), sputum induction, asthma control questionnaire (ACQ)[20](20), asthma quality life questionnaire (AQLQ)[21], and asthma severity as well as asthma control status in line with the GINA guidelines[2]. Then, a portable FeNO monitor (NObreath®; Bedfont Scientific Ltd., Rochester, Kent, UK), a peak flow monitor (PIKO®; nSpire Healt Ltd, London, UK), and an asthma diary were provided to all subjects in order to measure FeNO and PEF levels twice a day over two weeks at home. At the second visit when subjects brought the monitors back, the asthma diary was collected.

Spirometry and reversibility tests

Spirometry tests were performed with a dry wedge spirometer (Vitalograph, Buckingham, UK) at the first visit. Asthmatic subjects continued their usual medications except for salbutamol. FVC and FEV_1 levels before and after inhalation of 400μg salbutamol were recorded and the best of three acceptable maneuvers reproducible to within 200ml or 5% was retained[22].

FeNO and PEF measurements

At the first visit, all subjects were instructed on how to obtain measurements of FeNO and PEF, and to follow these measurements with taking their inhaled therapy.. FeNO measurements were conducted at a constant flow of 50ml/s in line with the ATS/ERS recommendations using a portable handheld analyzer (NObreath®)[23]. FeNO was performed twice a day for 2 weeks. Three successive measurements were performed on each occasion and levels were recorded in the diary. PEF measurements were also made three times after FeNO assessment. The best of the three readings was automatically recorded onto the monitor. All measurements over 2 weeks were transferred to a computer when subjects returned the monitor.

In order to avoid measurement bias, subjects were asked to measure FeNO and PEF at the same time and the same place twice a day, in the morning between 7 and 10am, and in the evening between 6 and 9pm. Subjects were advised not to take their usual medications prior to making the FeNO or PEF measurements. FeNO wasmeasured prior to PEF measurements.

Recording of asthma exacerbations

After the 2 weeks of measurements, patients were asked to keep a record of exacerbations over the subsequent 3 months, and were reviewed at 3 months. Severe exacerbations were defined as those needing treatment with oral prednisolone, or an increase in the maintenance dose of prednisolone for at least 3 or more days, or a hospitalization or emergency room visit because of asthma, requiring systemic corticosteroids. Moderate exacerbations were defined as 2 or more days with having one or more of the followings: deterioration in symptoms, deterioration in PEF levels, and increased rescue bronchodilator use, which is not severe enough to warrant systemic corticosteroid use [5].

Data analysis

Data are shown as means and 95% confidence interval (CI). The daily morning and evening FeNO levels were calculated as mean of two or three measurements. Diurnal variations of FeNO and PEF were expressed as the amplitude (highest-lowest). In addition, we used other ways of expressing the daily variation of these parameters in relation to their predictive value for uncontrolled asthma (Table 1). The diurnal variations of FeNO and PEF were compared between groups using Kruskall-Wallis and Mann-Whitney U-test with Bonferroni correction. Receiver operating characteristic (ROC) curves were constructed and the area under the curves (AUCs) were determined for the detection of uncontrolled asthma. Multivariate logistic regression analysis was performed to these 3 markers simultaneously in order to determine the independent predictors of uncontrolled asthma. Finally, the Chi-square test was used to evaluate the exacerbation rates between stable controlled and uncontrolled asthmatic subjects. A two-tailed *p* value of less than 0.05 considered significant.

RESULTS

Characteristics of subjects

Twenty-two subjects had stable controlled asthma and 28 had uncontrolled asthma (Table 2). Sputum eosinophils (%) in both stable and uncontrolled asthmatic groups were significantly higher compared to those in healthy group ($p<0.05$). There was a significant positive correlation between FeNO levels at the study entry and reversibility to salbutamol ($r=0.663$, $p<0.001$), and sputum eosinophil % ($r=0.370$, $p=0.014$). There was a negative correlation between FeNO levels at entry, FEV_1 %predicted ($r=-0.393$, $p=0.001$), and PEF %predicted ($r=-0.379$, $p=0.002$). There was no difference in the incidence of allergic rhinitis between stable controlled and uncontrolled asthmatic subjects.

Diurnal variation FeNO and PEF

Examples of individual diurnal variation in FeNO and PEF are shown in Figure 1. There was a significant diurnal FeNO variation (ΔFeNO(diurnal)) in uncontrolled asthmatic subjects (mean; 15.6ppb, 95CI; 12.5-18.7ppb) compared to stable controlled asthmatic (mean; 8.18ppb, 95CI; 6.69-9.67ppb) and healthy subjects (mean; 6.05ppb, 95CI; 5.19-6.90ppb) ($p<0.001$). On the other hand, no significant diurnal variation in PEF levels (ΔPEF(diurnal)) was observed in uncontrolled and stable controlled asthmatic subjects (mean; 39.4L/min, 95%CI; 30.3-48.5L/min vs mean; 34.5L/min, 95%CI; 29.1-39.9L/min) (Figure 2A and 2B). When the asthmatic groups were divided according to asthma severity, no significant differences in ΔFeNO(diurnal) and ΔPEF(diurnal) could be found between non-severe and severe groups, but there was a greater variation in these parameters between asthmatic groups and healthy subjects (Figure 2C and 2D). In addition, being on asthma medications such as oral prednisolone or theophylline did not influence the diurnal variation of FeNO levels.

Fluctuations of daily average FeNO and PEF levels over 2 weeks

The daily average FeNO levels in uncontrolled asthmatic subjects over 2 weeks were significantly higher than those in stable controlled asthmatic subjects (mean; 48.6ppb, 95% CI; 38.9-58.4ppb vs mean; 34.5ppb, 95% CI;

27.6-41.4ppb, p=0.03) and healthy subjects (mean; 48.6ppb, 95CI; 38.9-58.4ppb vs mean; 18.1ppb, 95%CI; 14.6-21.7ppb, p<0.001). In addition, the fluctuation pattern in uncontrolled asthmatic subjects was significantly different from those in stable controlled asthmatics and healthy subjects according to repeated ANOVA analysis (p<0.001) (Figure 3A), whereas the fluctuation pattern between stable controlled and healthy subjects showed no difference. When asthmatic subjects were divided into asthma severities, there was no significant difference in the daily average FeNO levels as well as the fluctuation pattern between non-severe and severe asthmatic group (Figure 3B).There was no significant difference in the fluctuation pattern of FeNO levels between morning and evening (data not shown).

Figure 4A shows the daily average levels of PEF based on asthma control status. The daily average PEF levels in uncontrolled asthmatic subjects were significantly lower than those in stable controlled asthmatic subjects (mean; 311L/min, 95CI; 277-346L/min vs mean; 415L/min, 95%CI; 377-452L/min, p<0.001) and healthy subjects (mean; 311L/min, 95CI; 277-346L/min vs mean; 513L/min, 95%CI; 482-544L/min, p<0.001). In addition, there were no significant fluctuated patterns in the daily average PEF levels over 2 weeks between healthy, stable controlled and uncontrolled asthmatic subjects (p>0.05). The similar results could be obtained when subjects were divided into healthy, non-severe asthmatic, and severe asthmatic subjects (Figure 4B). There was no difference in the fluctuation pattern of PEF levels between morning and evening (data not shown).

Asthma exacerbations

In uncontrolled asthmatic subjects group, 22 subjects (78.6%) experienced at least one more asthma exacerbations, whilst only 2 subjects (9%) in stable controlled asthmatic subjects exacerbated over the next 3 months after completed measurements (p<0.001) (Table 4A). Sixteen out of 24 asthmatic subjects (57.1%) with exacerbation had severe and 8 (36.3%) had non-severe asthma (Table 4B).

FeNO and PEF as predictor of control

ROC curves were constructed for predicting uncontrolled asthma using different pattern of calculations in FeNO and PEF levels (Table 3). In terms of FeNO levels, ∆FeNO (diurnal) was the best predictor for evaluating uncontrolled asthmatic subjects (AUC=0.803, sensitivity of 64.3%, specificity of 95.5%, positive predictive value (PPV) of 94.7% and negative predictive value (NPV) of 67.7%, p<0.001). With regard to PEF levels, the best parameter to estimate uncontrolled asthmatic subjects was PEF(Min%Max) (AUC=0.741, sensitivity of 60.7%, specificity of 81.8%, PPV of 81.0%, NPV of 62.1%, p<0.001) and %PEF(week) (AUC=0.741, sensitivity of 60.7%, specificity of 81.8%, PPV of 81.0%, NPV of 62.1%, p<0.001). ACQ score (AUC=0.772, sensitivity of 71.4%, specificity of 86.4%, PPV of 87.0%, NPV of 70.4%, p<0.001) was noted to predict uncontrolled asthmatic subjects.

Multiple logistic regression and ROC curves for predicting control

According to the multiple logistic regression analysis, ACQ scores, ΔFeNO(diurnal), and PEF(Min%Max) were found to be independent parameters for predicting uncontrolled asthmatic subjects (Table 5). Neither marker was superior according to the AUC curves, but the combination of ΔFeNO(diurnal) ≥16.6% and ACQ scores ≥ 1.8 was the best markers to detect uncontrolled asthmatic subjects with sensitivity of 85.7%, specificity of 86.4%, PPV of 88.9%, and NPV of 82.6% (AUC; 0.914 (95%CI: 0.857-0.971), p<0.001) (Figure 5).

DISCUSSION

In 50 patients with asthma subdivided according to asthma control, we found significant diurnal FeNO variations (ΔFeNO(diurnal)) in uncontrolled asthmatic subjects compared to stable controlled asthmatic and healthy subjects. In addition, the daily average FeNO levels, but not of PEF, in uncontrolled asthmatic subjects over 2 weeks showed a greater fluctuation than those in stable controlled asthmatic and healthy subjects. Severity of asthma was indicated by the raised level of baseline FeNO but not by the disturbance in diurnal variation. Finally, the combination of diurnal FeNO variation (ΔFeNO(diurnal)) and ACQ scores, rather than the use of each parameter separately, provides better information to predict uncontrolled asthma and future risks of asthma exacerbation.

FeNO has been shown to track with eosinophilic inflammation in the airways of patients with asthma [14]. Studies have also supported its role in reflecting asthma control[15;24;25] and as a marker of response to inhaled corticosteroid therapy[26;27]. However, several studies have shown its limitation in monitoring asthma therapy [28-30]. Support for a greater usefulness of daily FeNO measurements has recently been obtained[17;18;31;32]. In the current study, a single measurement of FeNO correlated well with FEV_1 % predicted and with percent sputum eosinophil indicating that FeNO reflected both the inflammatory component as well as airflow obstruction. A recent study showed that the level of asthma control was associated with inflammatory markers including FeNO and airway mucosal eosinophil numbers[33]. Our new findings indicate that the daily fluctuation and the diurnal variation of FeNO provided more information regarding the control of asthma and the future risk of exacerbation. Our data is in agreement with recent studies reporting that monitoring of FeNO revealed changes in FeNO prior to the onset of moderate exacerbations[32], and that analysis of the fluctuation of daily FeNO provided useful information on asthma control [17].

In our study, ΔFeNO(diurnal) but not ΔPEF(diurnal) in uncontrolled asthmatic subjects were significantly higher than those in stable controlled. There are reports indicating good relationships between diurnal PEF variations,

asthma control, and asthma exacerbation[6;34;35], while others report only limited usefulness as a measure of asthma control[36-38]. However, there are reports of significant diurnal variation in FeNO measurements in the mornings compared to evenings, as we have found[18;39]. In our study, we measured the fluctuation as the absolute variability irrespective of whether the level was higher or lower in any one direction. Frey and colleagues reported that the exponent of long-range fluctuation (α value) of FeNO and PEF predicted individual future risks of asthma exacerbation, and reflected the current state of asthma control[6;17;35;40] Our short-term analysis over only 2 weeks indicated that the diurnal fluctuation in FeNO was more related to asthma control than that of PEF. ΔFeNO(diurnal) rather than FeNO(daily), ΔFeNO(week) or %FeNO(week) appear to be a better marker for predicting asthma control and future risks of asthma exacerbation.

When ΔFeNO(diurnal) and ACQ scores are combined, the highest AUC could be obtained, whereas neither parameter seemed superior when used alone. In addition, these parameters are independent of each other from the multiple logistic regression analysis. There is no gold standard to estimate asthma control. What is clear is that no single parameter is adequate to assess asthma control and to predict future risks of exacerbation[5;41]. However, our result showed that most uncontrolled asthmatic subjects experienced asthma exacerbation over the next 3 months after measurements had been made, suggesting that asthma control could strongly influence the future risk of exacerbation, as reported previously[10-12].

One potential limitation of the study is that we did not assess adherence to treatment and because FeNO has been previously used to assess patient adherence [42] (44), our diurnal variation of FeNO may be a reflection of poor adherence. However, it is unlikely that there was poor adherence in the group of patients we studied. This was a short 2-week observational study and all participants provided all the measurements of FeNO and PEFR on a twice daily basis. Since they had to take their inhaler treatments after taking these measurements, we presumed that this happened as this would be their usual

treatments. One potential issue with the portable FeNO machine is its expense which will preclude its widespread use as a home monitoring device for an individual patient. However, if a 2-week monitoring period is sufficient to predict future risks of exacerbations and help to prevent these, then the value of the FeNO monitor would be increased.

In conclusion, diurnal variation as well as day-to-day variation in FeNO can be used as a surrogate biomarker of asthma control and as a predictor of the risk of future exacerbation. Further prospective studies are needed to clarify which markers could be combined to provide the best prediction for poor asthma control and future risks of exacerbation. Whether the daily and diurnal fluctuation of FeNO could be used to optimise asthma management should also be studied.

Acknowledgement

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Table1.

FeNO and PEF expressed in different ways in relation to their predictive values for uncontrolled asthma

Predictors	Formula
FeNO	
ΔFeNO(diurnal)	FeNO (Highest in a day - Lowest in a day)
FeNO(daily)	FeNO (morning + evening)/2
ΔFeNO(week)	FeNO (Highest over 2 weeks - Lowest over 2 weeks)
%FeNO(week)	FeNO (Highest over 2 weeks - Lowest over 2 weeks)/2weeks average
FeNO(Min%Max)	FeNO (Lowest over 2 weeks/Highest over 2 weeks)*100
PEF	
ΔPEF(diurnal)	PEF (Highest in a day - Lowest in a day)
PEF(daily)	PEF (morning + evening)/2
ΔPEF(week)	PEF (Highest over 2 weeks - Lowest over 2 weeks)
%PEF(week)	PEF (Highest over 2 weeks - Lowest over 2 weeks)/2weeks average
PEF(Min%Max)	PEF (Lowest over 2 weeks/Highest over 2 weeks)*100

FeNO: Fractional exhaled nitric oxide; PEF: Peak expiratory flow; ACQ: asthma control questionnaire.

Table 2. Subject characteristics

	Healthy non-asthma (n=15)	Stable controlled asthma (n=22)	Uncontrolled asthma (n=28)
Age (years)	36.2 (30.8-41.6)	42.3 (35.6-49.0)	51.4 (46.1-56.6)*
Sex (Male/Female)	10/5	8/14	10/18
Height (cm)	168 (164-173)	169 (165-173)	168 (164-171)
Weight (kg)	63.9 (54.5-73.2)	72.2 (65.3-79.2)	77.9 (70.4-85.4)*
BMI	22.3 (19.9-24.7)	25.3 (23.2-27.4)	27.8 (25.2-30.3)*
Atopy (%)	0	63.6*	85.7*
Allergic rhinitis (%)	0	54.5*	64.3*
Asthma severity	N/A	Non-severe; 15, Severe; 7	Non-severe; 7, Severe; 7
FEV_1 (%pred)	100 (93.9-107)	79.7 (73.4-86.1)*	66.8 (58.0-75.5)*†
FEV_1 /FVC (%)	83.7 (80.5-87.0)	72.0 (69.0-75.0)*	67.9 (62.6-73.2)*
Sputum eosinophils (%)	0.59 (0.20-0.98)	4.79 (1.82-7.76)*	18.5 (2.91-34.0)*†
Sputum neutrophils (%)	34.5 (22.2-46.9)	55.9 (41.8-69.9)	39.0 (21.1-56.8)
ACQ	N/A	1.41 (1.04-1.78)	2.52 (2.05-2.98)†
AQLQ	N/A	5.78 (5.40-6.16)	4.49 (4.00-4.99)†
Medication taken (no. (%))			
Inhaled corticosteroid	N/A	16 (72.7)	27 (96.4)†
Long-acting β_2 agonist	N/A	15 (68.2)	25 (89.3)†
Leukotriene antagonist	N/A	6 (27.3)	7 (25.0)
Theophylline	N/A	1 (4.54)	11 (39.3)†
Oral prednisolone	N/A	4 (18.2)	14 (50.0)†
Omalizumab	N/A	1 (4.54)	2 (7.1)
As-needed reliever use, inhalations of SABA /day	N/A	0.49 (0.07-1.06)	3.59 (2.34-4.84)†
ICS (BDP equivalent) (μg)	N/A	1009 (620-1398)	1804 (1543-2065)†

BMI: body mass index; ACQ: asthma control questionnaire; AQLQ: asthma quality of life questionnaire; BDP: beclomethasone dipropionate; FeNO: Fractional exhaled nitric oxide; FEV_1: Forced expiratory volume in one second; FVC: forced vital capacity; ICS: inhaled corticosteroid; N/A: not applicable; ppb: parts per billion; SABA: short acting β_2-agonist. Data shown as mean + 95% confidence interval. *$p<0.05$ vs healthy, † $p<0.05$ vs stable controlled asthmatic subjects.

Table 3. Diagnostic value of different parameters or combination of markers for predicting uncontrolled asthma

Predictors	AUC	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	p value
FeNO						
ΔFeNO(diurnal)	0.803 (0.715-0.891)	64.3	95.5	94.7	67.7	<0.001
FeNO(daily)	0.582 (0.470-0.695)	42.6	72.7	66.7	50	0.162
ΔFeNO(week)	0.789 (0.699-0.878)	67.9	86.4	86.4	67.9	<0.001
%FeNO(week)	0.721 (0.619-0.824)	85.7	54.5	70.6	75.0	<0.001
FeNO(Min%Max)	0.720 (0.615-0.825)	82.1	63.6	74.2	73.7	<0.001
PEF						
ΔPEF(diurnal)	0.527 (0.407-0.646)	35.7	100	100	55	0.659
PEF(daily)	0.735 (0.637-0.833)	57.1	77.3	76.2	58.6	<0.001
ΔPEF(week)	0.619 (0.505-0.733)	53.6	81.8	78.9	58.1	0.048
%PEF(week)	0.741 (0.641-0.840)	60.7	81.8	81.0	62.1	<0.001
PEF(Min%Max)	0.741 (0.641-0.840)	60.7	81.8	81.0	62.1	<0.001
ACQ						
ACQ score	0.772 (0.677-0.867)	71.4	86.4	87.0	70.4	<0.001
Combination						
ΔPEF(Min%Max)+ACQ	0.816 (0.733-0.900)	64.3	90.9	90.0	66.7	<0.001
ΔFeNO(diurnal)+ACQ	0.914 (0.857-0.971)	85.7	86.4	88.9	82.6	<0.001

FeNO: Fractional exhaled nitric oxide; PEF: Peak expiratory flow; ACQ: asthma control questionnaire. AUC shown as mean + 95% confidence interval.

Table 4.

A. Asthma control and exacerbations over 3 months follow-up

	Stable controlled asthmatic subjects	Uncontrolled asthmatic subjects	Total
Severe exacerbation	2 (9)	12 (42.9)	14
Moderate exacerbation	0 (0)	10 (35.7)	10
No exacerbation	20 (91)	6 (21.4)	26
Total	22 (100)	28 (100)	50

Data shown as subject numbers and percentage in brackets.
Chi-squared test: $p<0.001$

B. Asthma severity and exacerbations over 3 months follow-up

	Non-severe asthmatic subjects	Severe asthmatic subjects	Total
Severe exacerbation	3 (13.6)	11 (39.2)	14
Moderate exacerbation	5 (22.7)	5 (17.9)	10
No exacerbation	14 (63.6)	12 (42.9)	26
Total	22 (100)	28 (100)	50

Data shown as subject numbers and percentage in brackets.
Chi-squared test: p=N.S.

Table 5.

Predictors for discriminating uncontrolled asthma from stable controlled asthma using multiple logistic regression analysis

Predictors	Odds Ratio	**95% C.I.**	**P value**
ACQ scores	3.31	1.23-8.90	0.018
ΔFeNO (diurnal)	1.39	1.17-1.65	<0.001
PEF(Min%Max)	0.94	0.89-0.99	0.015
FEV_1 (%predicted)	0.99	0.94-1.04	0.62

ACQ: asthma control questionnaire; FeNO: fractional exhaled nitric oxide; PEF: peak expiratory flow; FEV_1: Forced expiratory volume in one second; C.I.: Confidence interval.

Figure legends

Figure 1:

Examples of typical individual patterns of FeNO and PEF levels over 2 weeks in healthy, stable controlled and uncontrolled asthmatic subjects. The horizontal axis shows time in days and the vertical axis indicates either FeNO levels or PEF levels. Morning FeNO and PEF levels are connected with solid lines, and evening FeNO and PEF levels with dotted lines.

Fig 1

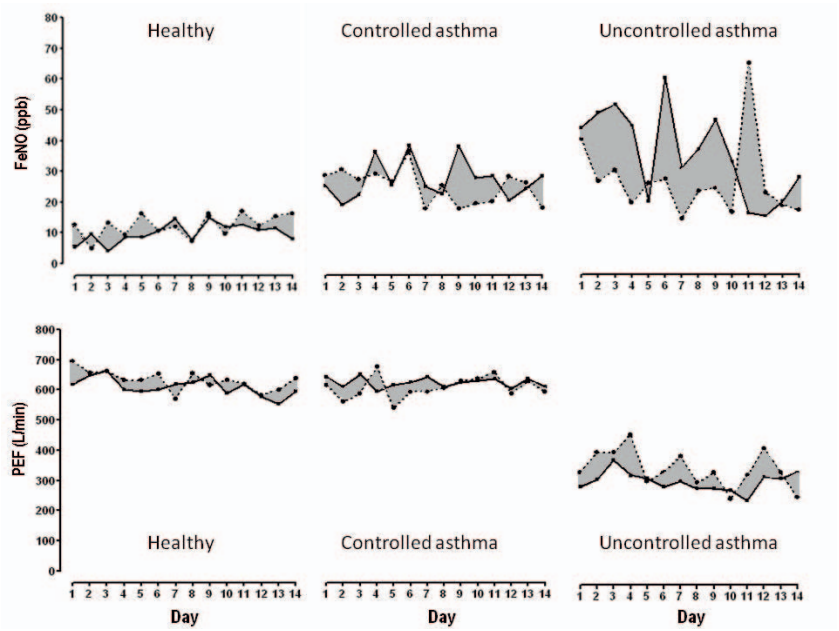


Figure 2:

Diurnal variations of FeNO (A,C) and PEF (B, D) levels in healthy and asthmatic subjects according to either asthma severity or asthma control are shown. *P≤0.05, ** P≤0.01, *** P≤0.001

Fig 2

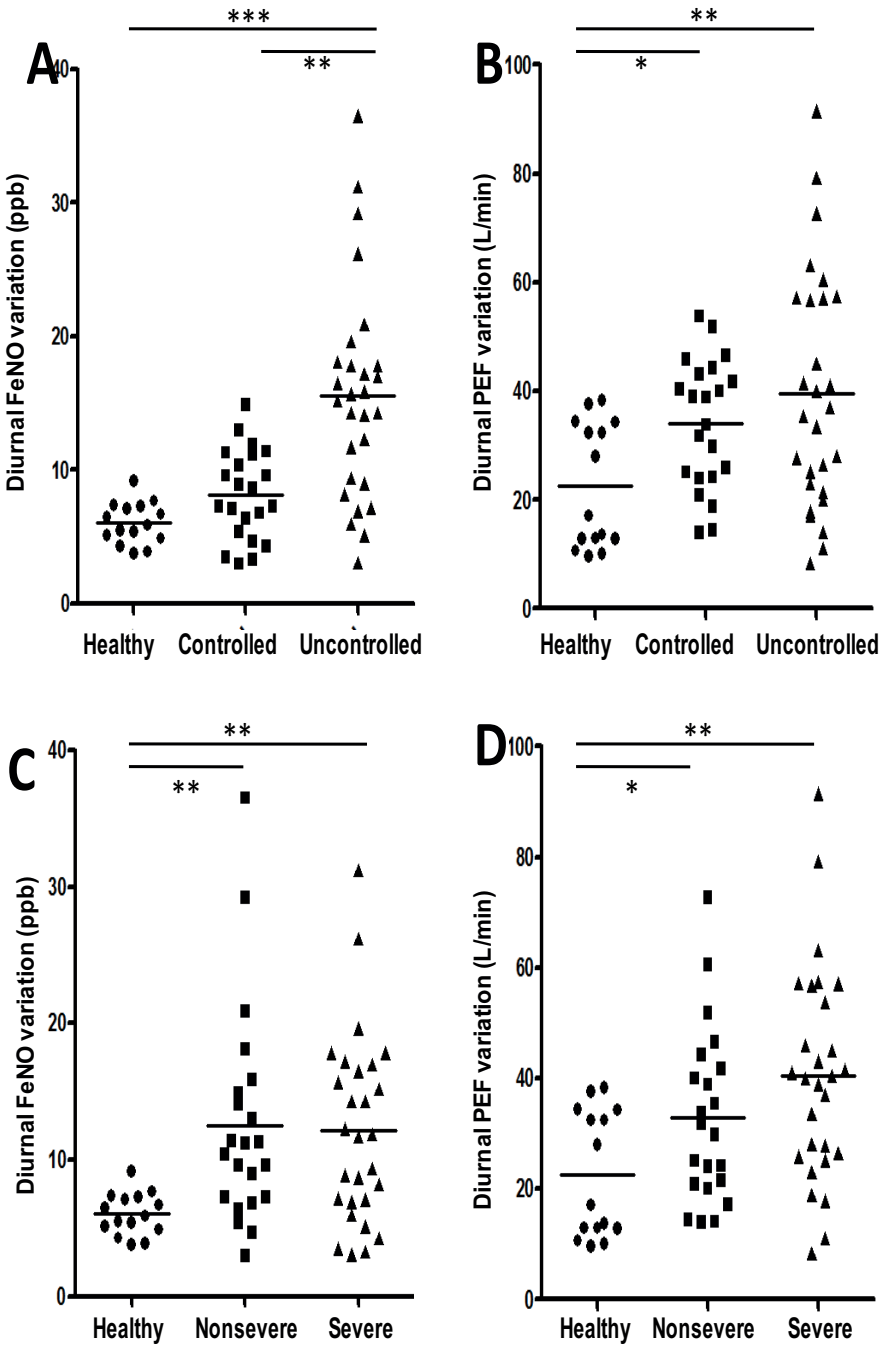


Figure 3:

Daily average variation of FeNO levels over 2 weeks according to asthma control status (A) or asthma severities (B) are depicted. The horizontal axis shows time in days and the vertical axis indicates FeNO levels. Uncontrolled asthmatic (solid diamonds), stable controlled asthmatic (solid squares), severe asthmatic (open diamonds), non-severe asthmatic (open squares), and healthy subjects (solid circles) are shown. Data are expressed mean and standarderror (SE). **p≤0.01 vs stable controlled asthmatic subjects and healthy subjects

Fig 3

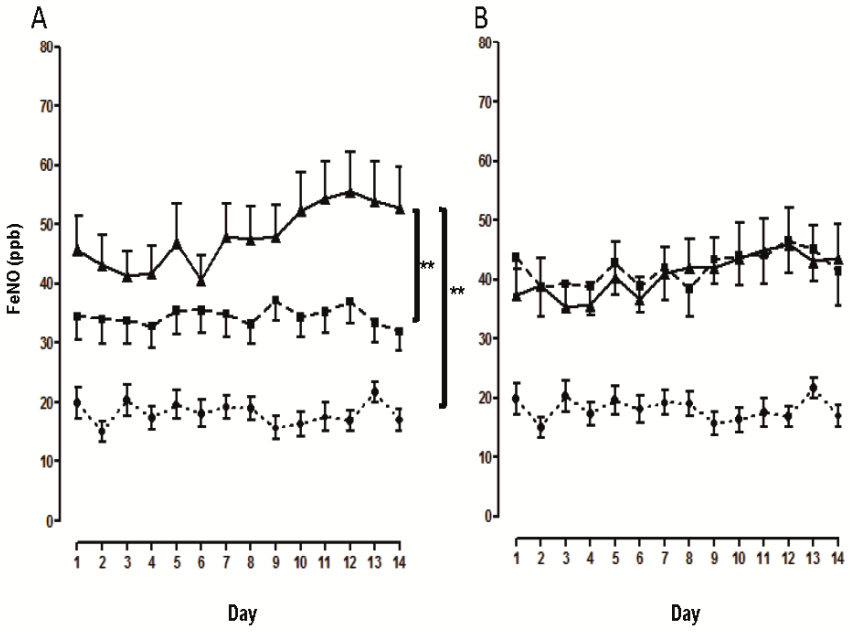


Figure 4:

Daily average variation of PEF levels over 2 weeks according to asthma control status (A) or asthma severities (B) are depicted. The horizontal axis shows time in days and the vertical axis indicates PEF levels. Uncontrolled asthmatic (solid diamonds), stable controlled asthmatic (solid square), severe asthmatic (open diamonds), non-severe asthmatic (open square), and healthy subjects (solid circles) are shown. Data are expressed mean and standard error (SE). **p≤0.01 vs stable controlled asthmatic subjects and healthy subjects

Fig 4

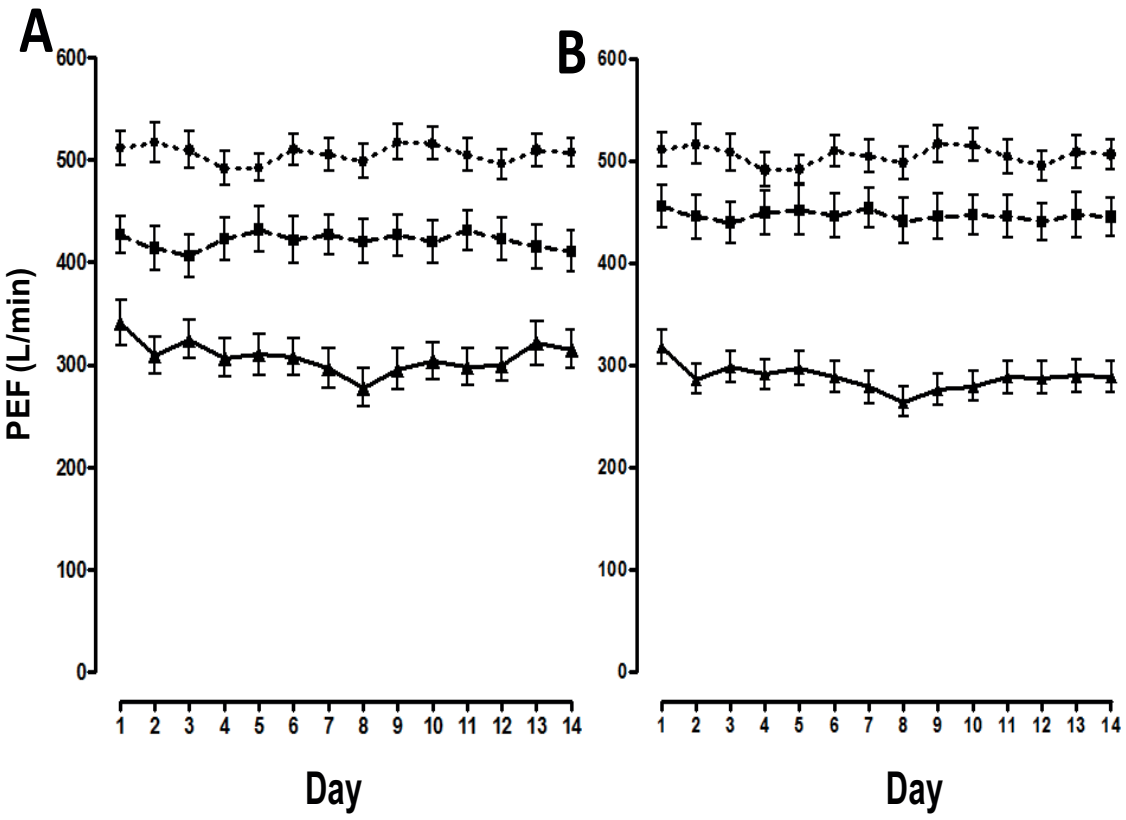
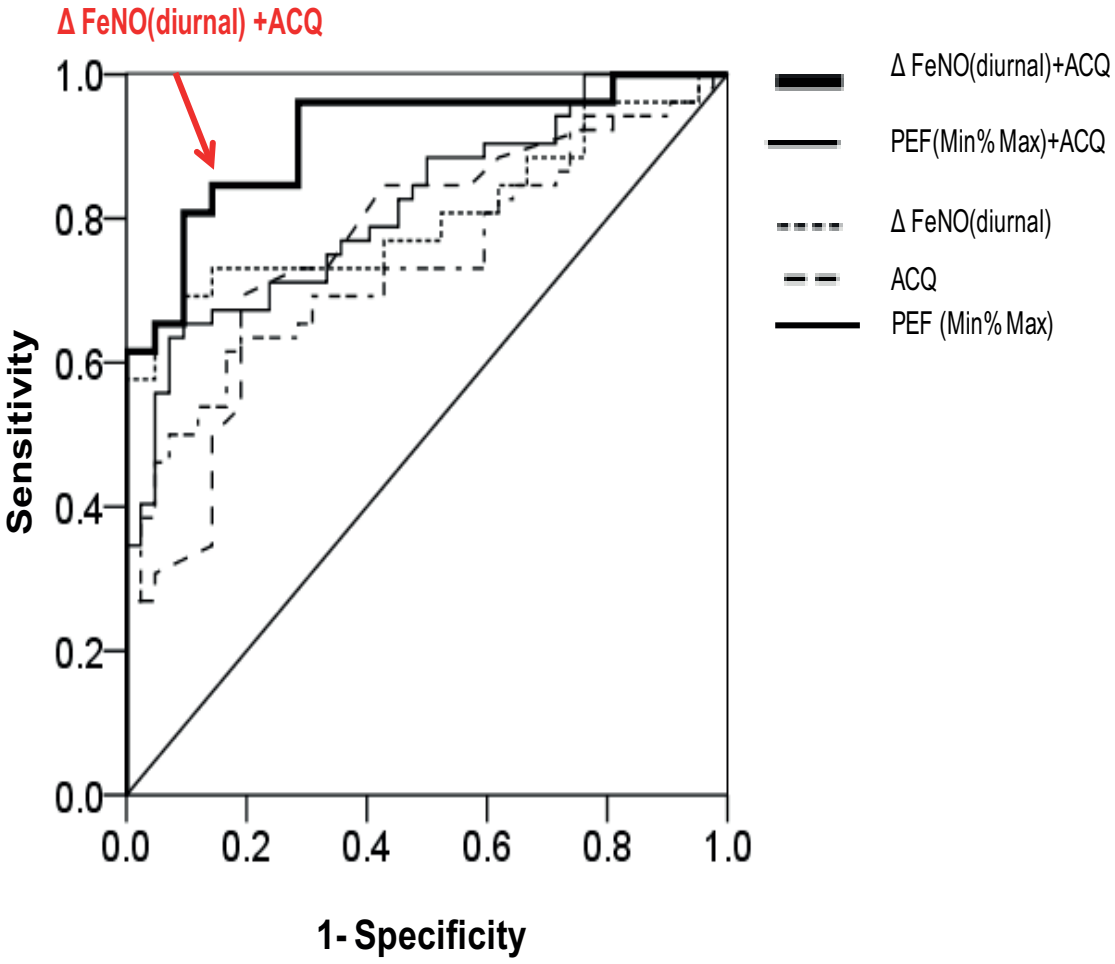


Figure 5:

Combined Receiver Operator Characteristics (ROC) curve for daily FeNO variation, PEF ratio and ACQ score. The combination of daily FeNO variation and ACQ score were the best parameters to differentiate uncontrolled asthmatic subjects from stable controlled asthmatic subjects (p<0.001).



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