ERJ Express. Published on December 8, 2009 as doi: 10.1183/09031936.00118809

Title: Exhaled nitric oxide predicts control in patients with difficult-to-treat asthma.

REVISED VERSION.

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Support statement:

GSK provided the exhaled nitric oxide analysers used in this work. The sources of funding did not participate in the collection, analysis and interpretation of the data, in the writing of the manuscript, nor in the decision to submit the manuscript for publication.

Abstract.

to evaluate the accuracy of baseline exhaled nitric oxide (F_eNO) levels to recognize individuals with difficult-to-treat asthma who have the potential to achieve control with a guidelines-based stepwise strategy.

One hundred two consecutive patients with suboptimal asthma control underwent with maximal fluticasone-salmeterol combination dose for one month. Then, those who remained uncontrolled received oral corticosteroids for an additional month.

With this approach, 53 patients (52%) gained control. Those who achieved control were more likely to have positive skin results (60.4 % vs 34 %; p = 0.01), positive bronchodilator test (57.1 % vs 35.8 %; p = 0.02) and peak expiratory flow variability ≥ 20% (71.1 % vs 49.1 %; p = 0.04). Conversely, depression was more frequent in those who remained uncontrolled (18.4 % vs 43.4 %; p = 0.01). An F_eNO value ≥ 30 ppb demonstrated a sensitivity of 87.5% (95% CI, 73.9 % to 94.5 %) and a specificity of 90.6% (95% CI, 79.7 % to 95.9 %) for the identification of responsive asthmatics.

The current results suggest that F_eNO can identify patients with difficult-to-treat asthma and the potential to respond to high doses of inhaled corticosteroids or systemic steroids.

Keywords:

Asthma control; difficult-to-treat; exhaled nitric oxide; salmeterol/fluticasone combination; severe asthma.

Introduction.

Asthma treatment ideally achieves a steady state of no symptoms and no exacerbations with minimum medication. In practice, many patients with severe disease will not be optimally controlled, and asthma surveys indicate that a high proportion of patients remain uncontrolled even while receiving adequate therapy[1,2]. Despite guideline-based asthma management goals, the Asthma Insights and Reality in Europe (AIRE) study of 2.803 european patients showed that 46% of them reported daytime symptoms, 30% had asthma-related sleep disturbances at least once a week, 25% has unscheduled urgent care visit in the past year, 10% had an emergency room visit and 7% had an overnight hospitalization[3].

There is no universally accepted definition of difficult-to-treat asthma. However, it is reasonable to consider it present when people have persistent symptoms and frequent exacerbations, despite being treated at steps 4 or 5 of GINA. Patients with difficult-to-treat asthma are a cause of concern because of impairment of quality of life, continued decreased lung function and adverse effects of high dose corticosteroids. Escalation of therapy to maximum doses of inhaled corticosteroids and oral corticosteroids is widely accepted in poorly controlled chronic asthmatics[4]. However, there is significant heterogeneity in the response to steroids and patients may be committed to an ineffective and potentially harmful therapy[5].

In the past years, asthma specialists have been interested in looking for an easy-to-measure and reliable biomarker that could facilitate the assessment of the disease and could avoid the risk of too little or too much treatment. Exhaled nitric oxide (F_eNO) –an indirect marker of eosinophilic airway inflammation, easy to perform and reproducible- has been successfully employed to guide asthma management[6-8].

This study was designed to focus on the practical problem of the patient with diagnosed asthma whose symptoms are not controlled despite high doses of inhaled corticosteroids and other regular therapy. In this prospective, observational study, we adjusted medication according to a stepwise approach, corresponding to the international guidelines, based on asthma control. The aim was to evaluate the accuracy of baseline exhaled nitric oxide F_eNO levels to recognize individuals with difficult-to-treat asthma who have the potential to achieve control with the stepwise approach. Control was defined as a score of greater than or equal to 20 in the Asthma Control test (ACT). A secondary objective was to identify determinants of asthma control among variables related to clinical and functional patient characteristics.

Methods.

Study design.

This was an observational and prospective study carried out in the pneumology and allergology units of one hospital. All patients were seen and assessed by the same physician at visit 1 and every subsequent clinical visit. The protocol was run on an outpatient basis.

Patients.

Consecutive patients were recruited for protocol evaluation between April 2008 and July 2009 if they had a difficult-to-treat asthma defined as not controlled asthma - ACT score < 20- despite minimal maintenance therapy of long acting β_2 agonist and high dose inhaled corticosteroids (≥ 800μg beclometasone equivalent) for > 3 months. All subjects had been free of exacerbations for, at least, this period. All of them were regularly followed at an outpatient asthma clinic and they were educated on the nature of the illness and the correct use of inhalers. The patients were advised to bring their maintenance mediation to the hospital and all of them denied nonadherence at the time

of the first clinical assessment. Patients treated with oral corticosteroids or omalizumab were excluded. We also excluded those with a smoking history in excess of 10 pack-years. Inclusion criteria were relatively broad because authors wanted to reflect usual clinical practice.

Protocol.

-First visit: Patients were classified according to their clinical control (assessed by the ACT). In all cases, medical history, spirometry, bronchodilator test, methacholine test (if not contraindicated), F_eNO, and ambulatory PEF measurement were recorded. A detailed structured clinical history was obtained for all subjects in order to rule out comorbid conditions (obesity, smoking, anxiety, depression, medications, oesophageal reflux, upper airways disease, vocal cord dysfunction, bronchiectasis, chronic obstructive pulmonary disease and allergic bronchopulmonary aspergillosis) that could influence asthma control. Vocal chord dysfunction was diagnosed with endoscopy when clinically suspected. Oesophageal reflux was diagnosed by means of medical history (pH-metry was not systematically performed). Patients with suspicion of bronchiectasis underwent high-resolution computed tomography. Anxiety and depression were assessed by use of Goldberg´s short questionnaire. Allergic bronchopulmonary aspergillosis was investigated in patients with high IgE levels.

Patients with suboptimal asthma control (ACT score < 20) with correct inhalation technique received increased therapy with maximal fluticasone-salmeterol combination dose (unless they were treated with such medication) and they were placed to return for a second visit one month later.

-Second visit: Patients with previous suboptimal asthma control who received increased therapy, were assessed for clinical control (ACT). If they achieved asthma control, a new spirometry and F_eNO measurement were performed. If they did not achieve

control, they underwent another increase of asthma therapy with 30 mg of deflazacort added to their inhaled medication and were placed to return for a third visit one month later.

-Third visit: Patients were assessed for asthma control. A new spirometry and F_eNO were recorded.

The time course of the protocol is shown in Figure 1.

Measurements.

Exhaled Nitric Oxide.

A single measurement was undertaken using a portable device (NIOX MINO; Aerocrine AB; Solna: Sweden) at a mouth flow rate of 50 ml/s during 10 seconds. The sensor on the device was changed periodically, in line with the manufacturer´s guidance.

Pulmonary function test.

Spirometry was performed using a Datospir 120 spirometer (Sibelmed, Spain) in accordance with American Society/European Respiratory Socienty guidelines[9] to determine forced expiratory volume in 1 second (FEV_1), forced vital capacity (FVC), FEV_1/FVC and peak expiratory flow (PEF). FEV_1 and FVC values are expressed as a percentage of predicted value (% pred), and changes in FEV_1 are expressed as a percentage of the initial value (Δ%). Bronchodilatador reversibility was calculated as the percentage of change in FEV_1 from baseline, 15 minutes after inhaling 400 µg of albuterol.

Airway hyperresponsiveness to methacholine (expressed as $PC_{20\ methacholine}$) was measured with a 2-min tidal breathing method adapted from Cockcroft and coworkers[10]. After an initial nebulized saline challenge, subjects inhaled doubling concentrations,

ranging from 0.038 to 19.2 mg/ml of methacholine-bromide, at 5-min intervals. Airway hyperresponsiveness to methacholine was defined as a $PC_{20} \leq 8$ mg/ml.

Diurnal peak expiratory flow rate (PEFR) was recorded in a daily record card.

Symptom assessment.

Asthma control was assessed using the validated Spanish version of the ACT[11]. Patients subjectively evaluated the degree of impairment caused by their asthma during the preceding 4 weeks by responding to five questions using a five-point-scale. The ACT is reliable, valid, and responsive to changes in asthma control over time. A cutoff score of 19 or less identifies patients with poorly controlled asthma[12].

Goldberg´s anxiety and depression test..

This test was designed to be used by non-psychiatrists in clinical investigations. The score is based on responses of 'yes' or 'no' to nine depression and nine anxiety items (the full set of nine questions need to be administered only if there are positive answers to the first four), asking how respondents have been feeling in the past month. Goldberg *et al.* (1988) considered patients with anxiety scores of 5 or more or with depression scores of 2 or more as having a 50% chance of a clinically important disturbance[13].

Statistical Analyses.

The baseline characteristics were compared between those who responded to increase of treatment with those who did not achieve control. Comparison of continuous data was made by Student´s *t* test or Wilcoxon signed rank test as appropriate. These tests where also applied for paired data when appropriate. Intergroup differences were evaluated by χ^2 analysis or Fisher's exact test for categorical data.

In order to determine the concentration of F_eNO capable of predicting asthma control after stepwise increase of therapy, we calculated sensitivity, specificity, positive predictive power, and negative predictive power, with their 95% confidence intervals

(CI), for each F_eNO measurement. With the resulting data, we generated a receiver operating characteristic (ROC) curve to find the best F_eNO cutoff (value with the greatest sensitivity and specificity) and to calculate the area under he curve (AUC), with its 95% CI, to estimate overall diagnostic accuracy. AUCs with a value of close to 1 indicated excellent ability to discriminate. A positive likelihood ratio [LR(+)] was calculated as sensitivity/(1 − specificity) or true-positive rate/false-positive rate. An LR(+) reflects increased odds of achieving control, after a positive F_eNO result. A negative likelihood ratio [LR(−)] is (1 − sensitivity)/specificity or false-negative rate/true-negative rate and reflects reduced odds of achieving asthma control after a negative F_eNO result. The ROC was fitted using the maximum likelihood fit of a binormal model by employing the web-based-calculator JROCFIT[14].

Logistic regression models were used to assess the relationship between predictor variables (duration of the disease, positive reactions to skin prick tests for common aeroallergens, clinical manifestations of atopic disease, presence of comorbidity, depression, BMI, F_eNO, FEV_1, daily PEF variability of 20% or more) and the event of interest (response to therapy).

A value of $p< 0.05$ was considered significant.

Statistical analysis were made using the R statistical package (R Development Core Team, 2009).

Results.

Patient demographics.

A total of 102 consecutive patients (mean age 56 ± 15 years, 71.6% females) with difficult-to-treat asthma were prospectively included from. At entry, mean ACT score was 14 ± 1. Baseline demographic and clinical characteristics are displayed in Table 1.

Mean FEV_1 at visit 1 was 72% and 50 % of the patients showed an obstructive pattern. Baseline F_eNO levels were 43.1 ± 45.6 ppb (range 4-222). Pulmonary function test are summarized in Table 2.

Consistent with the inclusion criteria, all patients were taking a combination of high-dose inhaled corticosteroid and long-acting β_2-agonist. A small proportion of the patients used additional supplementary treatments (Table 3).

Response to the stepwise increase of therapy.

At visit 2, 37 patients (36.2%) achieved control with the maximum dose of fluticasone/salmeterol combination. Of the remaining 65 patients, 16 (24.6%) gained control after one month of oral steroids (figure 1).

Only one patient suffered an exacerbation that required oral steroids, but not hospitalization, during the study. Another one was diagnosed of pneumonia (and admitted to hospital for this reason) while taking oral steroids between the second and the third visit.

Baseline characteristics for the two groups (patients who achieved control and those who did not) are listed in Table 1. Those patients who achieved control were more likely to have positive skin results (60.4 % vs 34 %; $p = 0.01$), positive bronchodilator test (57.1 % vs 35.8 %; $p = 0.02$) and PEF variability of more than 20% (71.1 % vs 49.1 %; $p = 0.04$). Conversely, depression was more frequent in the group of patients who remained uncontrolled (18.4 % vs 43.4 %; $p = 0.01$).

After applying the approach mentioned above, we have found that FEV_1 increased from 1.9 l/min ± 0.7 to 2.1 l/min ± 0.8 ($p < 0.01$), ACT increased from 14 (range 7-19) to 19 (range 7-25; $p < 0.01$) and F_eNO levels decreased from 28 (range 4-222) to 17.5 (range 4-94; $p < 0.01$).

At baseline, F_eNO was 67 ± 49 ppb in patients who finally achieved control, whereas it was 28 ± 36 ppb in those who did not gain control ($p< 0.0001$). At the end of the study, F_eNO was 32 ± 21 ppb in patients who achieved control and 16 ± 12 ppb in those who did not ($p< 0.0001$). Interestingly, F_eNO levels were above 35 ppb in 25% of the patients who gained control at the final visit.

Figure 3 shows the change in the median of ACT score and F_eNO value over time.

Predictive utility of F_eNO.

Exhaled nitric oxide was an excellent marker for predicting therapeutic response, with an area under the ROC curve (AUC) of 0.925. A F_eNO value of greater than 30 ppb demonstrated a sensitivity of 87.5% (95% CI, 73.9 % to 94.5 %) and a specificity of 90.6% (95% CI, 79.7 % to 95.9 %) for the identification of patients who will achieve control (Figure 2). The negative predictive value was 90.6 % (95% CI, 79.7 % to 95.9 %), and the positive predictive value was 87.5 % (95% CI, 73.9 % to 94.5 %). With a cutoff value of 30 ppb for F_eNO, the positive likelihood ratio was 9.3 (95% CI, 3.9 to 21.5) and the negative likelihood ratio was 0.14 (95% CI, 0-06 to 0.32).

Sensitivity, specificity, likelihood ratios and positive and negative predictive values at different cut off points of F_eNO values are showed in Table 4.

Factors associated with achievement of control.

On the basis of the univariate analysis, the following variables were introduced into the multivariate model: F_eNO, presence of comorbidity, positive skin prick testing, duration of the disease, BMI, FEV_1, PEF variability > 20% and depression. The multivariate analysis showed that only F_eNO (OR 47.7; 95% CI 13.9 to 163.9) independently and significantly correlated with the achievement of control.

Discussion.

Our results demonstrate, for the first time, that F_eNO levels might be predictive of response to a stepwise approach in patients with difficult-to-treat asthma. This study adds to previous research showing a clinical utility of F_eNO measurements in asthmatic patients[6-8]. It has been demonstrated that F_eNO correlates with eosinophilic inflammation measured using bronchial biopsies and induced sputum[15,16]. In addition, previous studies have shown that high numbers of sputum eosinophils were predictive of steroid response[17,18]. This underlines that steroid response is related to particular characteristics of airway inflammation. On the other hand, F_eNO is reduced by treatment with inhaled corticosteroids[19], but elevated levels of this biomarker were previously observed in patients with severe asthma despite corticosteroid treatment[20]. This might imply either steroid resistant inflammatory processes in the airway, or insufficient doses of anti-inflammatory medication. Theoretically, F_eNO measurements might help us to identify individuals with persistent eosinophilic inflammation in which a steroid response is more likely. This hypothesis is supported by our results in a difficult asthma population, indirectly by those of Smith et al[8], who found that F_eNO measurements provided a means of predicting steroid response in patients with undiagnosed respiratory symptoms, and also by the findings of Little et al[21], who have shown that response to oral steroids in asthma patients can be predicted in most cases by analyzing this biomarker.

Even with various expert-derived guidelines that provide asthma treatment strategies, many patients remain suboptimally controlled. In our series, 48 % of the patients did not achieve control –assessed by the ACT questionnaire- despite receiving the best available treatment and optimal management efforts. This figure is in accordance with the Gaining Optimal Asthma Control (GOAL) study[22]. It showed that symptoms were uncontrolled in as many as 38% of patients with moderate-to-severe asthma despite

high doses of salmeterol/fluticasone, good adherence (virtually 100%), and tightly monitored inhalation techniques. The addition of oral prednisolone (0.5 mg/Kg) led to a modest 7% increase in the percentage of well-controlled patients[22]. The most widely accepted explanation for these unsatisfactory findings is the view that the term "difficult-to-treat asthma" might include a broad spectrum of inflammatory patterns, not always as responsive to steroids as an eosinophil-associated process could be. In fact, several phenotypes of refractory asthma have been proposed, including those subjects who have persistent eosinophilic inflammation despite steroid treatment, but also those with predominant neutrophilic airway inflammation and those in whom virtually no inflammation is present on bronchial biopsy[23].

We have found a higher proportion of positive skin test results in those patients who achieved control than in those who remained uncontrolled. Although the classification between atopic and non-atopic disease has recently come under scrutiny, the ENFUMOSA study found fewer positive skin-prick tests in severe asthmatics compared with controlled patients, suggesting an association between atopy and the potential for poor/good asthma control with steroid/beta agonist therapy[24]. Positive bronchodilator test and PEF variability of more than 20% were also significantly more common in asthmatics who gained control, possibly reflecting a more reversible clinical situation. Conversely, depression was more frequent in patients who did not achieve control.

It must be noted that we assessed asthma control by administering the ACT. The recently published ATS/ERS consensus about standardization of outcomes relating to asthma control, recommend this kind of composite measures, designed to provide numeric comparisons of treatment effect[25]. This brief 5-item questionnaire measures several different areas of asthma control, including symptoms, rescue inhaler usage, and the impact of asthma in everyday functioning, but, even using this tool, accurate

assessment can be difficult and comorbidities might alter the scoring[26]. In two case series, coexisting disorders with asthma-like symptoms were found in 19% and 34% of patients with difficult asthma[27,28]. In such individuals, a variety of comorbid diseases, such as gastroesophageal reflux disease, obesity, vocal cord dysfunction and upper airway disease (e.g., seasonal allergies), may overlap with symptoms of asthma, making difficult to assess control. Particularly, it has been reported that depressive and anxiety disorders were associated with a decreased level of asthma control, including more visits to the doctor or emergency room, inability to do usual activities, and more days of symptoms compared to those without depression or anxiety[29]. We have not found differences in the rate of other comorbid conditions between patients who reached control and those who remained uncontrolled.

Some limitations of this study must be addressed. First, the sample size was small. Second, it is possible that the treatment periods (one month) were too short to reach the maximum effect. In fact, one study demonstrated that asthmatic patients with stable dosing tend to improve further, confirming the benefit of sustained treatment in subjects who have difficulty in achieving control[22]. Third, airway hyperresponsiveness –a factor that could potentially predict therapeutic response- was not assessed in all of the patients. Fourth, a selection bias is possible because the study design excluded patients who were taking oral steroids. Thus, the sample might not accurately represent the whole population of difficult-to-treat asthmatics. On the other hand, it must be taken into account that the diagnosis of severe asthma still represents a challenge for physicians, and many patients with other entities like COPD could be categorized as "difficult-to-treat asthma". However, in our sample there were 71% females, more than 90% were never smokers, hyperresponsiveness was present in almost all of them and mean FeNO value was 43 ppb. All of these facts, taken together, make us feel confident

that our patients were truly asthmatics. Fifth. the investigators were not blinded to the F_eNO results. This fact could be a possible source of bias, although we believe that the influence in our results is not relevant because therapeutic decisions were not based on F_eNO values bur rather were derived from the ACT score, which is self completed by the patients. Sixth, although all of the patients had been regularly followed at an outpatient asthma clinic, they had previously been educated on the correct use of inhalers, they were advised to bring their maintenance mediation to the hospital and all of them denied nonadherence at the time of the first clinical assessment we did not measure adherence objectively. Gamble et al[30] have recently demonstrated that a significant proportion of patients with difficult-to-control asthma remained nonadherent to inhaled or oral corticosteroids. However, patients were unaware that they were being observed and it is well known that patients who agree to participate in research are more likely than nonparticipants to be adherent with their regimen. Anyway, although noncompliance could underestimate the population response to the stepwise approach, it is unlikely to affect the predictive accuracy of FeNO.

Finally, it must be highlighted that no single outcome measure can adequately assess asthma control. The clinical value of composite scores like ACT is limited bay the lack of validation in a wider range of settings, particularly in patients with different asthma phenotypes.

The present study may have implications for clinical practice and future research. Such information could be beneficial when advising patients what to expect when deciding to escalate their medication and to employ potentially harmful drugs. On the other hand, it is of critical importance to identify patients who are less responsive to steroid treatment and are at risk of developing persistent airway obstruction. These patients should be closely monitored and considered for novel anti-asthma drugs in order to prevent

progression of the disease. In addition, attempts at treating by phenotype will aid in the development of a more rational approach to the evaluation of interventions like therapy with omalizumab, mepolizumab, imatimib or anti-tumor necrosis factor-alpha agents.

In conclusion, the current results suggest that F_eNO can identify patients with difficult-to-treat asthma and the potential to respond to high doses of inhaled corticosteroids or systemic steroids.

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Figure 1. Flow-chart that summarizes the protocol.

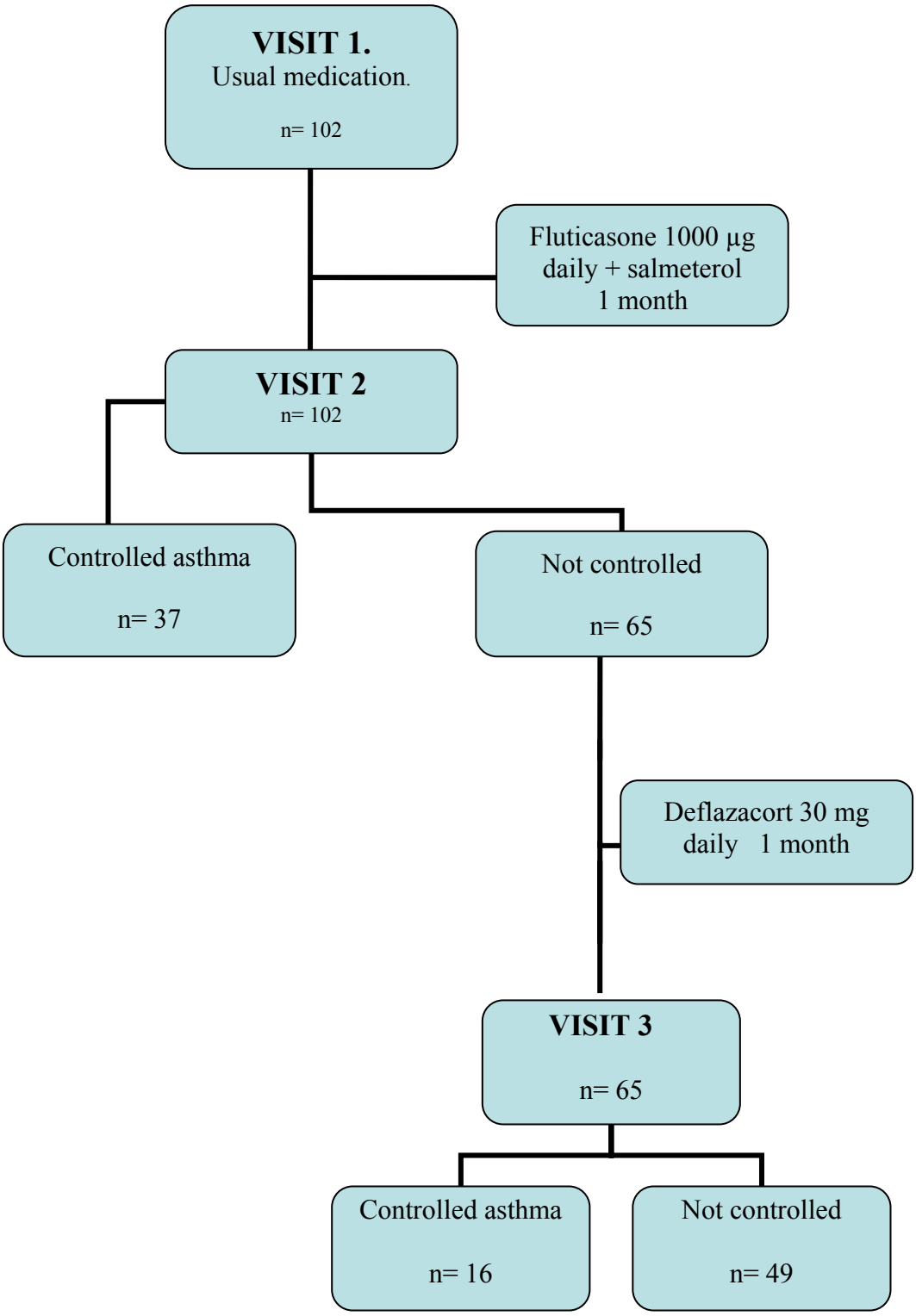
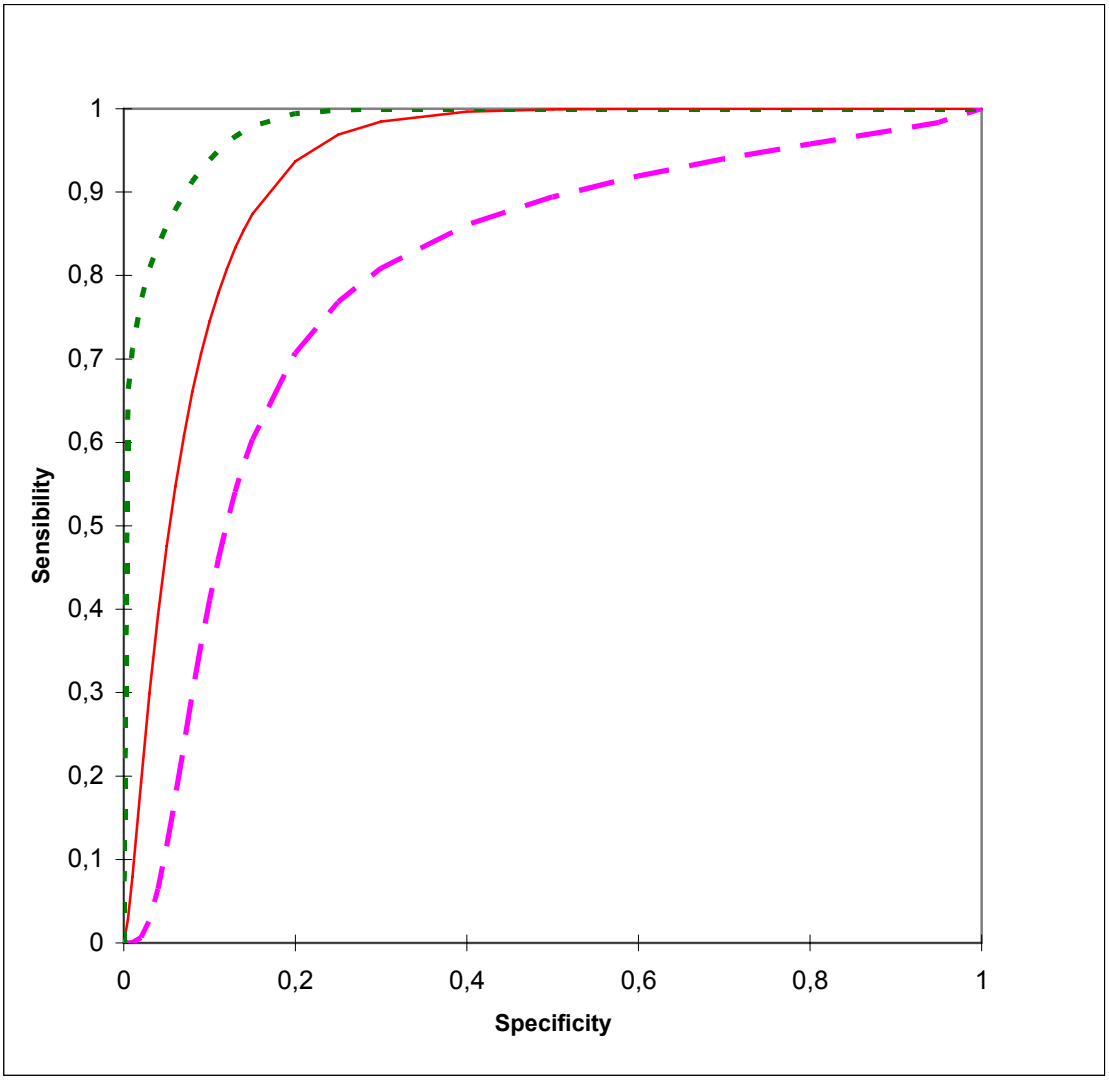
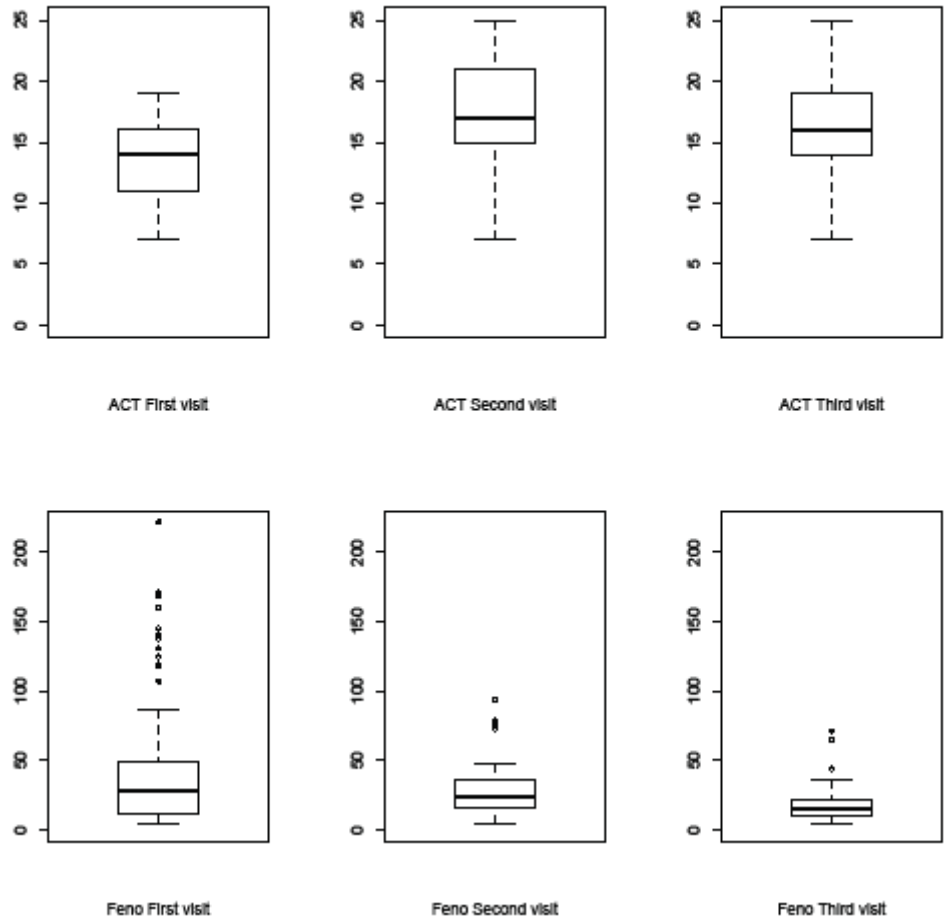


Figure 2. ROC curve for the prediction of therapeutic response from F_eNO measurements.



ROC curve identified the optimal cutoff value of 30 with 87.5% sensitivity (95% CI, 73.9 % to 94.5 %) and 90.6% specificity (95% CI, 79.7 % to 95.9 %) Area under the ROC curve is 0.925.

Figure 3. Box plots of ACT score and F_eNO values in successive visits.



Horizontal lines in boxes represent median values. Box ends at quartiles Q_1 and Q_3: whiskers extend to 5th and 95th percentiles; outliers are shown individually. ACT score increased significantly from visit 1 to visit 2. F_eNO value decreased significantly from visit 1 to visit3. It must be noted that median ACT score was higher at visit 2 than at visit 3. This apparent contradiction is explained by the fact that many patients achieved control (ACT > 20) at visit 2. However, at visit 3, the majority of subjects remained uncontrolled (ACT < 20). On the other hand, median F_eNO decreased at every visit reflecting that patients who did not achieve control showed low values of the biomarker.

Table 1. Study subjects´ baseline demographic and clinical characteristics by asthma control.

	All subjects	Controlled	Uncontrolled	Controlled vs Uncontrolled
Age, yr	56 ± 15	53 ± 17	58 ± 13	NS, p = 0.11
Females	72 (71%)	23 (67%)	39 (74%)	NS, p = 0.59
BMI	29.04 ± 6	27.95 ± 6.58	29.66 ± 5.37	NS, p = 0.16
Current smoker	7 (6.9%)	3 (6.1%)	4 (7.5%)	NS, p = 0.72
Duration of the disease, yr	20 ± 15	19 +- 14	25 +-16	NS, p = 0.07
Onset of the disease **-Childhood** **-Adult**	20 (19.8%) 82 (80.2%)	10 (20.4%) 40 (79.6%)	10 (19.2%) 42 (81.8%)	NS, p = 0.30
Positive skin test	47 (46.5%)	29 (60.4%)	18 (34%)	P = 0.01
Allergic comorbidities	70 (69.3%)	35 (71.4%)	35 (67.3%)	NS, p = 0.67
Aspirin intolerance	8 (8%)	2 (5.0%)	6 (11.8%)	NS, p = 0.26
Polyposis	9 (8.8%)	5 (12.5%)	4 (7.5%)	NS, p = 0.42
ABPA	10 (9.8%)	4 (8.2%)	6 (11.3%)	NS, p = 0.74
Gastroesophageal reflux	23 (228%)	39 (20.4%)	39 (25%)	NS, p = 0.58
Vocal cord dysfunction	3 (2.9%)	1 (2%)	2 (3.8%)	NS, p = 1.0
Anxiety	16 (15.7%)	7 (14.3%)	9 (17.0%)	NS, p = 0.70
Depression	32 (31.4%)	9 (18.4%)	23 (43.4%)	P = 0.01
ACT score	14 ± 1	14 ± 3	12 ± 3	P < 0.01

Data are presented as No (%), mean ± SD

Table 2. Pulmonary function test.

	All subjects	Controlled	Uncontrolled	Controlled vs Uncontrolled
FEV1, %	72 ± 24	75 ± 20	69 ± 27	NS, p = 0.18
FEV1/FVC < 70%	51 (50%)	25 (49%)	26 (51%)	NS, p = 1.0
+ bronchodilator test	47/99	30 (57.1%)	17 (35.8%)	NS, p = 0.02
PEF variability > 20%	58 (59%)	32 (71.1%)	26 (49.1%)	P = 0.04
+ methacholine test	69/71	35 (72.9%)	34 (66.7%)	NS, p = 0.37
FeNO (ppb)	43.1 (4-222)	68.4 (11-222)	19.7 (4-160)	P < 0.01

Data are presented as No (%), mean ± SD. F_eNO is expressed as median (range).

Table 3. Treatment at baseline.

Treatment	Nº of patients
BF (640/9)	**44 (43.1%)**
FS (1000/100)	**21 (20.5%)**
FS (500/100)	**27 (26.4%)**
Tiotropium bromide	**7 (6.8%)**
Montelukast	**39 (38.2%)**
Theophyline	**3 (2.9%)**

BF: Budesonide / formoterol daily maintenance dose

FS: Fluticasone / salmeterol daily maintenance dose

Table 3. Sensitivity, specificity, likelihood ratios and positive and negative predictive values at different cut off points of F_eNO values.

	Sensitivity % (IC 95%)	Specificity % (IC 95%)	+ Likelihood ratio	- Likelihood ratio	PPV	NPV
FENO ppb						
20	90 (76.9, 96.0)	81,1 (68.6, 89.4)	4.77 (2.70, 8.42)	0.12 (0.05, 0,32)	78.3 (64.4, 87.7)	91.5 (80.1, 96.6)
25	90 (76.9, 96.0)	84.9 (72.9, 92.1)	5.96 (3.12, 11.39)	0.12 (0.05, 0.30)	81.8 (68.0, 90.5)	91.8 (80.8, 96.8)
30	87.5 (73.9, 94.5)	90.6 (79.7, 95.9)	9.28 (3.99, 21.53)	0.14 (0.06, 0.32)	87.5 (73.9, 94.5)	90.6 (79.7, 95.9)
35	77.5 (62.5, 87.7)	90.6 (79.7, 95.9)	8.22 (3.51, 19.23)	0.25 (0.14, 0.45)	86.1 (71.3, 93.9)	84.2 (72.6, 91.5)
40	70.0 (54.6, 81.9)	94.3 (84.6, 98.1)	12.37 (4.04, 37.81)	0.32 (0.20, 0.51	90.3 (75.1, 96.7)	80.6 (69.1, 88.6)
45	67.5 (52.0, 79.9)	94.3 (84.6, 98.1)	11.93 (3.89, 36.55)	0.34 (0.22, 0.54)	90.0 (74.4, 96.5)	79.4 (67.8, 87.5)
50	42,5 (28.5, 57.8)	94.3 (84.6, 98.1)	7.51 (2.36, 23.87)	0.61 (0.46, 0.81)	85.0 (64.0, 94.8)	68.5 (57.1, 78.0)

PPV = Positive predicted value.

PPV= Negative predictive value.