Respiratory Medicine (2011) 105, 526–532

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/rmed

Exhaled NO and exhaled breath condensate pH in the evaluation of asthma control

Konstantinos Kostikas [a,*], Andriana I. Papaioannou [a], Kalliopi Tanou [a], Paschalina Giouleka [b], Angela Koutsokera [a], Markos Minas [a], Spyros Papiris [b], Konstantinos I. Gourgoulianis [a], D. Robin Taylor [c], Stelios Loukides [b]

[a] Respiratory Medicine Department, University of Thessaly Medical School, Larissa, Greece
[b] 2nd Respiratory Medicine Department, University of Athens Medical School, Athens, Greece
[c] Dunedin School of Medicine, University of Otago, Dunedin, New Zealand

Received 26 May 2010; accepted 13 October 2010
Available online 3 November 2010

KEYWORDS
Asthma control;
Exhaled NO;
Exhaled breath condensate pH;
Inhaled corticosteroids;
Smoking

Summary
Background: Asthma is a chronic inflammatory airways disorder. However, no biomarker of airways inflammation has been included in the assessment of asthma control.
Objective: To evaluate exhaled NO (FeNO) and exhaled breath condensate (EBC) pH in patients with asthma according to the level of control, and their performance in the identification of not well-controlled patients.
Methods: FeNO and EBC pH after Argon deaeration were measured in 274 consecutive patients. Asthma control was evaluated by two asthma specialists blinded to FeNO and pH measurements according to GINA guidelines, as well as by asthma control test (ACT) and asthma control questionnaire (ACQ).
Results: FeNO was higher and EBC pH was lower in patients with not well-controlled compared to controlled asthma. In ROC analysis, FeNO presented an AUC of 0.790 for the identification of not well-controlled asthma performing better in non-smokers; EBC pH presented an AUC of 0.791 for the identification of not well-controlled asthma, performing better in smokers. The performance of both biomarkers was inferior to that of ACT and ACQ. FeNO values >30 ppb presented positive predictive values (PPV) > 0.85 with the exception of smokers treated with inhaled corticosteroids. EBC pH values ≤ 7.20 presented PPV >0.80 in all groups. The presence of FeNO >30 ppb and/or EBC pH ≤ 7.20 was indicative of not well-uncontrolled asthma in 88.3% of the patients.
Conclusion: FeNO and EBC pH levels may identify patients with not well-controlled asthma. However, their performance was inferior to clinical judgment and may be limited to selected subgroups of asthmatic patients.

* Corresponding author. Stamouli 3, Karditsa 43100, Greece. Tel.: +30 6944780616; fax: +30 2441022370.
E-mail address: ktk@otenet.gr (K. Kostikas).

0954-6111/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.rmed.2010.10.015

Introduction

Asthma is by definition a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role.[1] Current asthma guidelines have focused on asthma control, both for the classification and for the proper management of individual patients.[1,2] The classification of patients according to asthma control requires an evaluation of symptoms, limitations in activities, use of rescue medication, history of exacerbations and pulmonary function testing.[1] In addition, the National Heart Lung and Blood Institute have proposed the use of validated questionnaires,[2] including the asthma control test (ACT)[3] and the asthma control questionnaire (ACQ).[4] However, despite the central role of inflammation in the pathogenesis and natural history of asthma, no biomarker of airways inflammation has been recommended in the assessment of control in current asthma guidelines.

The relation between symptoms and airways inflammation remains a controversial issue, especially in complex asthma.[5] Symptom perception may be influenced by several factors, including obesity[6] and the coexistence of anxiety and depression,[7] and the inaccurate perception of asthma symptoms is highly variable within persons.[8] Therefore, there is a need for objective evaluation of asthma symptoms and control, suggesting a possible role for biomarkers of airway inflammation. This may further facilitate management decisions, since the presence or absence of inflammation may be used to guide treatment modifications.[9,10]

The fraction of exhaled nitric oxide (FeNO) has been widely evaluated as a marker of eosinophilic airways inflammation, since it is readily measured, it provides reproducible results, and is responsive to changes in inhaled corticosteroid (ICS) doses.[11] The ability of FeNO in identifying well-controlled asthma has been evaluated in children[12] and in longitudinal studies in adults.[13] However, several confounding factors have been described in the evaluation of FeNO, including smoking and treatment with ICS.[13,14]

Exhaled breath condensate (EBC) pH has been found to decrease in acute asthma and resolves with treatment,[15] whereas stable patients with moderate asthma present lower pH compared to mild disease.[16] EBC pH is decreased in smokers compared to non-smokers with allergic rhinitis.[17] Further, smoking patients with moderate to severe asthma receiving ICS presented lower EBC pH values compared to non-smokers,[18] suggesting a possible role of smoking in EBC pH regulation. Besides FeNO, EBC pH represents the only exhaled biomarker to date that can be measured on-site and that has been shown to be reproducible and robust.[19]

The aim of this study was to evaluate the relationships between two non-invasive, easy-to-perform on-site, exhaled biomarkers (FeNO and EBC pH) in asthmatic patients according to their level of control. Secondary aims were to evaluate the diagnostic performance of FeNO and EBC pH for the identification of not well-controlled (partly controlled or uncontrolled) asthma in specific phenotypes of asthmatic patients, according to treatment with inhaled corticosteroids and their smoking habit.

Methods

Study participants

Patients with a previously established diagnosis of asthma that were evaluated in the outpatient asthma clinics of two tertiary University hospitals between January 2007 and August 2008 were included in the study. Exclusion criteria were: patients with a recent exacerbation (e.g. requiring hospitalization or oral corticosteroids); patients with another or coexisting respiratory disorder (e.g. COPD, bronchiectasis); patients with recent smoking cessation (<2 months prior to the study) given that FeNO levels return to normal levels 4–8 weeks after smoking cessation[20] in order to stratify our subjects as current smokers or non-smokers; patients who had been previously included in this study.

The study protocol was approved by the Ethics Committees of both hospitals and participants provided written informed consent.

Study design details

Study participants sequentially undertook FeNO measurement, EBC collection, spirometry with a dry spirometer (KoKo Legend, Ferraris, UK) according to ATS recommendations,[21] and evaluation by respiratory physicians. FeNO and EBC measurements were performed at 10:00–13:00 a.m. Subjects had not consumed food or beverages and had not smoked for 2 h before.

Evaluation of asthma control

Asthma control was evaluated according to GINA guidelines[1] by two respiratory physicians with special interest in asthma (K.K. and S.L.) who were blinded to FeNO and EBC pH measurements. Patients were classified as having "well-controlled", "partly controlled" or "uncontrolled" asthma, according to GINA. Patients with partly controlled and uncontrolled asthma were grouped as having "not well-controlled" asthma. In addition, two validated questionnaires, the Asthma Control Test (ACT)[3] and Juniper's Asthma Control Questionnaire (ACQ)[4] were used.

Measurement of exhaled NO

FeNO was measured using a portable NO analyzer (NIOX MINO® Airway Inflammation Monitor, Aerocrine, Solna, Sweden).[22] MINO measurements were performed at an expiratory flow rate of 50 mL/s, and are expressed as parts per billion (ppb). Measurements with this portable analyzer have previously been shown to be in clinically acceptable agreement with measurements provided by a stationary analyzer according to the ATS guidelines.[23–25]

Collection and measurement of exhaled breath condensate pH

EBC was collected using a commercially available device (EcoScreen, Viasys, Germany).[17] Subjects rinsed their mouth with distilled water and performed tidal breathing

for 15 min while wearing a nose clip. EBC pH was measured using a commercially available pH meter (Model 3510, Jenway, Essex, UK), immediately after the collection of condensate.[16] Stable pH was achieved after deaeration of the EBC with argon (350 mL/min for 10 min).[16]

Statistical analysis

Normally distributed data are presented as mean ± SD, skewed data as median (interquartile ranges), and categorical data as *n* (%). Comparisons between groups were performed with Kruskall–Wallis tests with Dunn's post-hoc tests. For the assessment of FeNO and EBC pH performance as predictors of asthma control, receiver operating characteristic (ROC) curves were created. Areas under the ROC curves (AUC) with 95% confidence intervals (CI) and their differences from 0.5 were calculated. Sensitivities, specificities, positive (PPV) and negative (NPV) predictive values were calculated for the optimal cut-points. Statistical analysis was performed with GraphPad Prism 5 (GraphPad Software Inc, La Jolla, CA, USA) and MedCalc 9 (MedCalc Software, Mariakerke, Belgium).

Results

Demographics and parameters related to asthma control in the 274 patients who were included are presented in Table 1. Patients were additionally divided into four subgroups according to their smoking status and their use of ICS: Group 1 – ICS-untreated non-smokers ($n = 48$); Group 2 – ICS-untreated smokers ($n = 32$); Group 3 – ICS-treated non-smokers ($n = 144$); and Group 4 – ICS-treated smokers ($n = 50$).

FeNO and EBC pH values according to asthma control

Exhaled NO and exhaled breath condensate (EBC) pH values in the whole population and in the 4 subgroups are presented in Table 2. Patients with partly controlled asthma had higher FeNO and lower EBC pH values compared to those with well-controlled asthma. This was evident in the whole study population and also in groups 1, 2 and 3. Additionally, patients with uncontrolled asthma had higher FeNO and EBC pH values compared to those with partly or well-controlled asthma in the groups of non-smokers (groups 1 and 3). In group 2, FeNO and EBC pH values in patients with uncontrolled asthma were statistically significant different from those with well-controlled asthma, but did not differ from those with partly controlled disease. Finally, in group 4 (ICS-treated smokers) there were no statistically significant differences between the three groups in either FeNO or EBC pH.

Diagnostic performance of FeNO and EBC pH for the identification of not well-controlled asthma

The diagnostic performance of FeNO, EBC pH and their combination (i.e. the presence of either one of the two biomarkers) for the identification of patients with not well-controlled (i.e. partly controlled or uncontrolled) asthma is presented in Table 3 and the corresponding ROC curves are shown in Fig. 1. For the whole study population, the performance characteristics for both FeNO and EBC pH, as judged by the AUCs, were significant (>0.7) but modest (<0.9). FeNO provided a PPV of 0.89 at a cut-point of >24 ppb, whereas EBC pH provided a PPV of 0.81 for a cut-point of ≤ 7.37. However, the diagnostic performance of both biomarkers and their combination was inferior to those of ACQ (AUC 0.880, 95% CI 0.835 to 0.916) or ACT (0.918, 95% CI 0.879 to 0.948) (Fig. 1).

In group 1 the diagnostic performance of FeNO was better compared to EBC pH or the combination of the two biomarkers (AUC = 0.899, PPV = 0.90 at a cut-point of >22 ppb). In Group 2, in contrast, the diagnostic performance of EBC pH was superior to that of FeNO, yielding a PPV of 0.85 at a cut-point of ≤ 7.21, as was the combination of the two biomarkers. In Group 3, the diagnostic performance was comparable for both biomarkers and their combination, with FeNO providing a PPV of 0.95 for values >27 ppb. Finally, in Group 4, the diagnostic performance was poor for both biomarkers. In this group the combination of the two biomarkers improved their diagnostic performance.

Evaluation of the diagnostic performance of FeNO and EBC pH at different cut-points

In the evaluation of different cut-off points we observed that FeNO values >30 ppb provide PPV >0.90 for the

Table 1 Patients' demographics and parameters related to asthma control.

	All ($n = 274$)	Well controlled ($n = 99$)	Partly controlled ($n = 115$)	Uncontrolled ($n = 60$)
Age (years)	50 ± 17	51 ± 18	51 ± 17	46 ± 15
Gender (F/M)	166/109	64/35	68/47	36/24
BMI (kg/m^2)	27.9 ± 4.9	28.5 ± 4.7	27.5 ± 5.1	28.0 ± 5.0
Smokers (%)	83 (30.3%)	31 (31.3%)	33 (28.7%)	19 (31.7%)
FEV_1 (% pred.)	85 ± 19	94 ± 17	83 ± 16	74 ± 21
ICS-treated (%)	194 (70.8%)	67 (67.7%)	91 (79.1%)	36 (60.0%)
ACQ	1.43 (0.71–2.71)	0.57 (0.29–0.86)	1.86 (1.14–2.71)	3.43 (2.57–4.00)
ACT	19 (16–23)	23 (22–24)	18 (17–19)	14 (11–17)

Normally distributed data are presented as mean ± SD; skewed data are presented as median (interquartile ranges); and categorical data as n (%). BMI: body-mass index; FEV_1: forced expiratory volume in 1 s; ICS: inhaled corticosteroids; ACQ: asthma control questionnaire (Juniper); ACT: asthma control test.

Table 2 Exhaled NO and exhaled breath condensate (EBC) pH values in the whole population and in the 4 subgroups.

		All (n = 274)	Well controlled (n = 99)	Partly controlled (n = 115)	Uncontrolled (n = 60)
All (n = 274)	FeNO	22 (16–41)	16 (13–20)	27 (19–44)*	59 (23–111)*,#
	EBC pH	7.29 (7.14–7.43)	7.44 (7.34–7.57)	7.25 (7.12–7.36)*	7.14 (7.05–7.21)*,#
Group 1 (n = 48)	FeNO	30 (18–111)	16 (14–21)	40 (27–105)*	116 (63–145)*,#
	EBC pH	7.25 (7.16–7.40)	7.43 (7.27–7.45)	7.25 (7.19–7.34)*	7.11 (7.08–7.22)*,#
Group 2 (n = 32)	FeNO	19 (14–22)	16 (12–19)	21 (15–38)*	22 (21–108)*
	EBC pH	7.32 (7.14–7.47)	7.47 (7.40–7.58)	7.19 (7.12–7.33)*	7.15 (7.03–7.21)*
Group 3 (n = 144)	FeNO	23 (16–44)	16(12–20)	28 (20–44)*	61 (35–78)*,#
	EBC pH	7.31 (7.15–7.45)	7.48 (7.34–7.59)	7.29 (7.12–7.36)*	7.15 (7.08–7.21)*,#
Group 4 (n = 50)	FeNO	19 (14–25)	17 (14–22)	19 (13–25)	23 (17–74)
	EBC pH	7.24 (7.10–7.41)	7.38 (7.26–7.53)	7.21 (7.06–7.41)	7.11 (6.96–7.54)

Data are presented as median (interquartile ranges). FeNO values are expressed in ppb at a flow rate of 50 mL/s *$p < 0.05$ compared to controlled asthma; #$p < 0.05$ compared to partly controlled asthma. Group 1: ICS-untreated – Non-Smokers; Group 2: ICS-untreated – Smokers; Group 3: ICS-treated – Non-Smokers; Group 4: ICS-treated – Smokers.

identification of not well-controlled asthma, both in the whole population and in the two groups of non-smokers (Groups 1 and 3, Table 4 Online supplement). However, this was at the cost of low NPV, suggesting that low FeNO values are not useful for the identification of well-controlled asthma.

Additionally, EBC pH values ≤7.20 provide PPV >0.80 for the identification of not well-controlled asthma (Table 5 Online supplement). In contrast, the higher PPVs in the groups of smokers (0.84 for Group 2 and 0.86 for Group 4) may suggest a possible role for low EBC pH for the identification of not well-controlled asthma in asthmatic smokers. Again, this was at the cost of low NPV, suggesting that high values of EBC pH are not useful for the identification of well-controlled asthma.

A scatterplot of FeNO and EBC pH levels of individual patients according to their level of control is presented in Fig. 2. The majority of well-controlled patients are classified in the lower right quartile of the graph (FeNO <30 and EBC pH >7.20). Only 16 of 99 well-controlled patients (16.2%) had either FeNO values of >30 and/or EBC pH ≤7.20. Additionally, 121 of the 137 patients (88.3%) that presented with partly controlled or uncontrolled asthma had FeNO >30 and/or EBC pH ≤7.20.

Diagnostic performance of FeNO and EBC pH for the differentiation between uncontrolled and partly controlled asthma

The diagnostic performance of both biomarkers (and their combination) for the differentiation between uncontrolled and partly controlled asthma was poor, and this was even more prominent in smokers (data not shown).

Discussion

To our knowledge this is the first study to assess two completely non-invasive exhaled biomarkers for the

Table 3 Diagnostic performance characteristics of exhaled NO, EBC pH and their combination for the identification of not well-controlled (partly or uncontrolled) asthma in the whole study population and in the 4 subgroups. Data for the optimum cut-points based on maximum AUCs with ROC analyses, are given.

	Cut-point	Sensitivity	Specificity	PPV	NPV	AUC (95%CI)	p-value
All (n = 274)	FeNO >24 ppb	0.61	0.87	0.89	0.56	0.790 (0.737–0.837)	<0.0001
	pH ≤7.37	0.85	0.66	0.81	0.71	0.791 (0.738–0.837)	<0.0001
	Combination	0.93	0.66	0.81	0.71	0.754 (0.699–0.804)	<0.0001
Group 1 (n = 48)	FeNO >22 ppb	0.87	0.81	0.90	0.76	0.899 (0.778–0.967)	<0.0001
	pH ≤7.36	0.91	0.63	0.83	0.77	0.734 (0.587–0.851)	0.004
	Combination	0.97	0.50	0.79	0.89	0.734 (0.587–0.851)	<0.0001
Group 2 (n = 32)	FeNO >19 ppb	0.56	0.75	0.69	0.63	0.680 (0.492–0.833)	0.059
	pH ≤7.21	0.69	0.88	0.85	0.74	0.795 (0.616–0.916)	0.0003
	Combination	0.81	0.75	0.76	0.80	0.781 (0.600–0.907)	0.0007
Group 3 (n = 144)	FeNO >27 ppb	0.64	0.94	0.95	0.60	0.844 (0.775–0.899)	<0.0001
	pH ≤7.39	0.92	0.65	0.82	0.83	0.836 (0.765–0.892)	<0.0001
	Combination	0.93	0.63	0.82	0.85	0.785 (0.709–0.849)	<0.0001
Group 4 (n = 50)	FeNO >23 ppb	0.45	0.87	0.89	0.41	0.597 (0.449–0.733)	0.256
	pH ≤7.25	0.66	0.80	0.89	0.50	0.682 (0.535–0.806)	0.036
	Combination	0.77	0.73	0.87	0.58	0.752 (0.610–0.863)	0.003

CI: confidence intervals, PPV: positive predictive value, NPV: negative predictive value, AUC: area under the ROC curve. Group 1: ICS-untreated – Non-Smokers; Group 2: ICS-untreated – Smokers; Group 3: ICS-treated – Non-Smokers; Group 4: ICS-treated – Smokers.

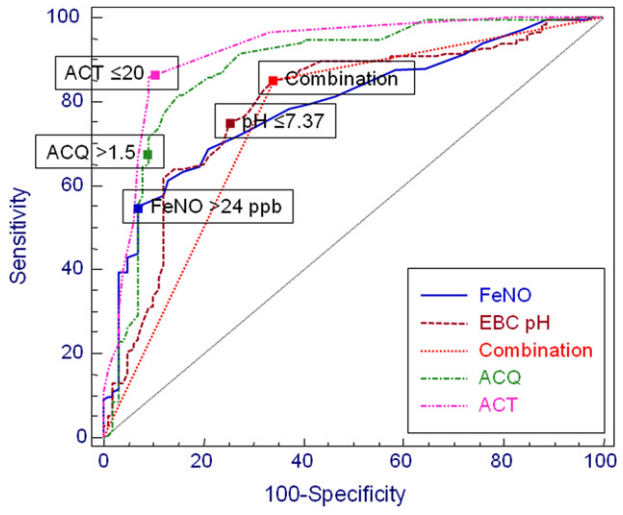

Figure 1 Receiver operating characteristics (ROC) curves for FeNO, EBC pH and their combination in the identification of not well-controlled asthma in the whole study population. Squares (■) indicate the optimal cut-off points for FeNO (>24 ppb), EBC pH (≤7.37), their combination, ACQ (≥1.5) and ACT (≤20).

evaluation of asthma control in an unselected outpatient population of asthmatics. We have shown that both FeNO and EBC pH, two easily performed on-site exhaled biomarkers, are related to the level of asthma control as defined in the GINA guidelines.[1] We have also observed that FeNO is helpful for the identification of not well-controlled asthma, performing better in non-smokers, whereas EBC pH was more effective in the identification of not well-controlled asthma in smokers. A complementary role for the two biomarkers in the identification of not well-controlled asthmatics in the

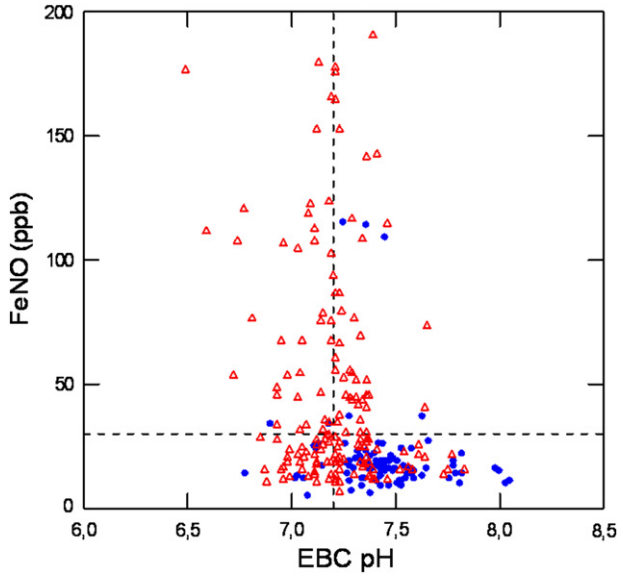

Figure 2 Scatterplot of the whole study population according to the level of asthma control: well controlled (●) vs. partly controlled or uncontrolled (▲). The dashed lines represent the optimal cut-points for FeNO (30 ppb) and EBC pH (7.20), according to ROC analysis.

group of smokers receiving ICS was observed. The presence of FeNO >30 and/or EBC pH ≤7.20 was indicative of partly or uncontrolled asthma in 88.3% of the patients. However, the performance of FeNO, EBC pH and their combination was inferior to clinical judgment and ACQ or ACT in the whole population.

Biomarkers of airway inflammation are under evaluation for the guidance of asthma management. Studies have shown that treatment strategies aiming at normalization of induced sputum eosinophils may reduce exacerbations,[9] especially eosinophilic ones,[26] without the need for additional anti-inflammatory treatment.[9,26] However, sputum induction is time-consuming and requires a dedicated laboratory. In contrast, FeNO is non-invasive, is measured in a few minutes and is responsive to changes in inhaled corticosteroid doses.[11] However, studies using FeNO guidance for asthma treatment have been controversial,[27,28] and this may reflect differences in design and the fact that cut-points used may reflect values from a normal population instead of the population of interest.[11]

Our study adds to this field as it provides data on the levels of FeNO that may be of clinical importance in the assessment of asthma control in everyday clinical practice. In a recent study Michils et al. showed that FeNO values >45 ppb exclude a well-controlled asthma with an NPV of 0.89.[13] In a similar manner, we have shown that FeNO values >30 ppb are suggestive of not well-controlled asthma with PPVs >0.80, with better performance in non-smokers (PPVs >0.90). Differences in study design may account for the lower cut-off point in our study, since in our study we did not exclude smokers and asthma control was prospectively evaluated by a chest physician, in contrast to the study by Michils et al. where asthma control was evaluated by a post-hoc analysis of ACQ data. The performance of both ACQ and ACT was superior to that of FeNO, EBC pH and their combination in our study, yet it was inferior and cannot substitute for the clinical evaluation by clinicians with special interest in asthma.

The interpretation of FeNO has to take account of several confounding factors, two of the most important being smoking habit and the use of ICS. A recent study has shown that sequential changes in FeNO are related to asthma control even in smokers.[14] Our present data clearly show that this is not the case for the cross-sectional assessment of single FeNO measurements in smoking asthmatics. In contrast, we have shown that EBC pH may be of greater importance in smokers, as evaluated by the corresponding AUCs in Groups 2 and 4 of our study. Especially in smokers not treated with ICS at the time of the interpretation, EBC pH presents an acceptable performance (AUC = 0.795) with a PPV of 0.85 for values ≤7.21. Moreover, the bad performance of both single biomarkers in Group 4 may be partly compensated by using the combination of the two, providing an AUC of 0.752.

EBC pH has never been previously evaluated in the assessment of asthma control. The rationale for the use of EBC pH lies in the fact that patients with acute asthma present lower pH values that resolve with treatment.[15] We have previously shown that pH values in stable patients with mild asthma do not differ from controls, whereas patients with moderate asthma have lower pH values.[16] Additionally, patients on ICS have higher EBC pH values that do not differ

from controls.[16] In a recent study we have highlighted a possible role for smoking in the regulation of endogenous airways acidification in patients with allergic rhinitis.[17] The present data further support our previous observations and suggest that patients with partly or uncontrolled asthma present with lower EBC pH values compared to those with well-controlled disease. An additional role of smoking was evident in Group 2 patients (ICS-untreated smokers) but was eliminated in Group 4 (ICS-treated smokers). However, even in that group, EBC pH remained a better biomarker compared to FeNO for the identification of not well-controlled patients. This may be attributed both to the fact that smoking asthmatics present lower FeNO values compared to non-smokers[29] and to a possible association of lower EBC pH with neutrophilic airways inflammation, as we have previously shown for patients with COPD or bronchiectasis.[16] Smoking has been shown to lead to an alteration of the inflammatory pattern in favor of a more neutrophilic type of inflammation in asthmatics.[30] Finally, EBC pH has been related to the levels of oxidative stress in smokers with moderate to severe asthma,[18] further supporting a possible role of EBC pH in confirming true loss of control in such patients, as suggested by our data.

In the evaluation of different cut-points for the identification of not well-controlled asthma we have shown that a single value of FeNO $>$30 ppb provides PPVs $>$0.90 in the non-smoking asthmatics, however at the cost of low NPV. This is in accordance with previous studies showing that high FeNO values present high specificity and low sensitivity for the diagnosis of asthma.[22,31] Our results are similar, yet in the present study we have chosen to evaluate cut-points with high PPV (i.e. those that provide minimal risk to miss a patient with not well-controlled asthma),[32] since our study population is representative of the patients evaluated in asthma clinics. Interestingly, and despite the low diagnostic performance of FeNO in Group 4, values $>$30 ppb provided a PPV of 0.83, suggesting that high FeNO values are indicative of poor asthma control even in this subgroup of ICS-treated smoking patients.

The evaluation of different cut-off points for EBC pH revealed that values $\leq$7.20 provide PPVs $>$0.80 in the whole population and in all subgroups. The corresponding PPVs for the groups of smokers (Groups 2 and 4) were 0.84 and 0.86, suggesting that only a small fraction of patients with not well-controlled asthma will have lower values. This is the first study to our knowledge that evaluates cut-points of EBC pH for the identification of asthma control. It has to be pointed out that, in a similar manner to FeNO, this is at the cost of low NPVs, suggesting that EBC pH is not useful for the identification of well-controlled patients. However, in clinical practice the major need is to identify not well-controlled patients, since these are the patients where an intervention is urgently needed. Inaccurate perception of symptoms is often found in asthma patients[8] and especially in certain groups, including obese patients,[6] persons with psychosocial problems[7] and adolescents.[33] Interestingly, adolescents with asthma are at higher risk for depression, cigarette smoking and drug abuse[34] that may lead to poorer adherence to medication.[35] Therefore, the need for biomarkers that accurately identify patients with not well-controlled asthma is imperative, especially in certain subgroups of asthmatic patients, and FeNO and EBC pH may provide useful information in non-smokers and smokers, respectively.

In conclusion, we have shown that FeNO and EBC pH levels may be useful in the identification of patients with not well-controlled asthma. The performance of FeNO was better in non-smokers and in patients not receiving ICS, whereas EBC pH performed better in smokers. A complementary role for the two biomarkers was demonstrated in smokers treated with ICS. However, their performance was inferior to clinical judgment and may be limited to selected subgroups of asthmatic patients. Further longitudinal studies for the prospective evaluation of the performance of the two biomarkers to guide the management asthmatic patients are clearly justified.

Acknowledgments

The authors wish to thank Drs Eleni Karetsi, Agori Tsaroucha and Parthena Mystridou, as well as Ms Ioanna Charitou, for their invaluable help with the collection of data throughout the study.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.rmed.2010.10.15.

Conflicts of interest statement

D.R. Taylor has received two lecture fees and an unrestricted research grant from Aerocrine AB, a manufacturer of nitric oxide analyzers. All other authors declare that they have no conflict of interest related to the content of this manuscript.

References

1. Bateman ED, Hurd SS, Barnes PJ, Bousquet J, Drazen JM, Fitzgerald M, et al. Global strategy for asthma management and prevention: GINA executive summary. *Eur Respir J* 2008; **31**:143—78.
2. National Asthma Education and Prevention Program. *Expert Panel Report 3 (EPR3): guidelines for the diagnosis and management of asthma: full report*. Bethesda, MD: National Heart, Lung, and Blood Institute; 2007.
3. Nathan RA, Sorkness CA, Kosinski M, Schatz M, Li JT, Marcus P, et al. Development of the asthma control test: a survey for assessing asthma control. *J Allergy Clin Immunol* 2004;**113**: 59—65.
4. Juniper EF, O'Byrne PM, Guyatt GH, Ferrie PJ, King DR. Development and validation of a questionnaire to measure asthma control. *Eur Respir J* 1999;**14**:902—7.
5. Tillie-Leblond I, Montani D, Crestani B, de Blic J, Humbert M, Tunon-de-Lara M, et al. Relation between inflammation and symptoms in asthma. *Allergy* 2009;**64**:354—67.
6. Lessard A, Turcotte H, Cormier Y, Boulet LP. Obesity and asthma: a specific phenotype? *Chest* 2008;**134**:317—23.
7. Deshmukh VM, Toelle BG, Usherwood T, O'Grady B, Jenkins CR. The association of comorbid anxiety and depression with

asthma-related quality of life and symptom perception in adults. *Respirology* 2008;**13**:695—702.
8. Janssens T, Verleden G, De Peuter S, Van Diest I, Van den Bergh O. Inaccurate perception of asthma symptoms: a cognitive-affective framework and implications for asthma treatment. *Clin Psychol Rev* 2009;**29**:317—27.
9. Green RH, Brightling CE, McKenna S, Hargadon B, Parker D, Bradding P, et al. Asthma exacerbations and sputum eosinophil counts: a randomised controlled trial. *Lancet* 2002;**360**:1715—21.
10. Taylor DR, Pijnenburg MW, Smith AD, De Jongste JC. Exhaled nitric oxide measurements: clinical application and interpretation. *Thorax* 2006;**61**:817—27.
11. Gibson PG. Using fractional exhaled nitric oxide to guide asthma therapy: design and methodological issues for asthma treatment algorithm studies. *Clin Exp Allergy* 2009;**39**:478—90.
12. Robroeks CM, van de Kant KD, Jobsis Q, Hendriks HJ, van Gent R, Wouters EF, et al. Exhaled nitric oxide and biomarkers in exhaled breath condensate indicate the presence, severity and control of childhood asthma. *Clin Exp Allergy* 2007;**37**:1303—11.
13. Michils A, Baldassarre S, Van Muylem A. Exhaled nitric oxide and asthma control: a longitudinal study in unselected patients. *Eur Respir J* 2008;**31**:539—46.
14. Michils A, Louis R, Peche R, Baldassarre S, Van Muylem A. Exhaled nitric oxide as a marker of asthma control in smoking patients. *Eur Respir J* 2009;**33**:1295—301.
15. Hunt JF, Fang K, Malik R, Snyder A, Malhotra N, Platts-Mills TA, et al. Endogenous airway acidification. Implications for asthma pathophysiology. *Am J Respir Crit Care Med* 2000;**161**:694—9.
16. Kostikas K, Papatheodorou G, Ganas K, Psathakis K, Panagou P, Loukides S. pH in expired breath condensate of patients with inflammatory airway diseases. *Am J Respir Crit Care Med* 2002; **165**:1364—70.
17. Tanou K, Koutsokera A, Kiropoulos TS, Maniati M, Papaioannou AI, Georga K, et al. Inflammatory and oxidative stress biomarkers in allergic rhinitis: the effect of smoking. *Clin Exp Allergy* 2009;**39**:345—53.
18. Kane B, Borrill Z, Southworth T, Woodcock A, Singh D. Reduced exhaled breath condensate pH in asthmatic smokers using inhaled corticosteroids. *Respirology* 2009;**14**:419—23.
19. Vaughan J, Ngamtrakulpanit L, Pajewski TN, Turner R, Nguyen TA, Smith A, et al. Exhaled breath condensate pH is a robust and reproducible assay of airway acidity. *Eur Respir J* 2003;**22**:889—94.
20. Hogman M, Holmkvist T, Walinder R, Merilainen P, Ludviksdottir D, Hakansson L, et al. Increased nitric oxide elimination from the airways after smoking cessation. *Clin Sci (Lond)* 2002;**103**:15—9.
21. Standardization of Spirometry. 1994 Update. American Thoracic Society. *Am J Respir Crit Care Med* 1995;**152**:1107—36.
22. Kostikas K, Papaioannou AI, Tanou K, Koutsokera A, Papala M, Gourgoulianis KI. Portable exhaled nitric oxide as a screening tool for asthma in young adults during pollen season. *Chest* 2008;**133**:906—13.
23. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. *Am J Respir Crit Care Med* 2005;(171):912—30.
24. Alving K, Janson C, Nordvall L. Performance of a new handheld device for exhaled nitric oxide measurement in adults and children. *Respir Res* 2006;**7**:67.
25. Menzies D, Nair A, Lipworth BJ. Portable exhaled nitric oxide measurement: comparison with the "gold standard" technique. *Chest* 2007;**131**:410—4.
26. Jayaram L, Pizzichini MM, Cook RJ, Boulet LP, Lemiere C, Pizzichini E, et al. Determining asthma treatment by monitoring sputum cell counts: effect on exacerbations. *Eur Respir J* 2006;**27**:483—94.
27. Smith AD, Cowan JO, Brassett KP, Herbison GP, Taylor DR. Use of exhaled nitric oxide measurements to guide treatment in chronic asthma. *N Engl J Med* 2005;**352**:2163—73.
28. Szefler SJ, Mitchell H, Sorkness CA, Gergen PJ, O'Connor GT, Morgan WJ, et al. Management of asthma based on exhaled nitric oxide in addition to guideline-based treatment for inner-city adolescents and young adults: a randomised controlled trial. *Lancet* 2008;**372**:1065—72.
29. Verleden GM, Dupont LJ, Verpeut AC, Demedts MG. The effect of cigarette smoking on exhaled nitric oxide in mild steroid-naive asthmatics. *Chest* 1999;**116**:59—64.
30. Chalmers GW, MacLeod KJ, Thomson L, Little SA, McSharry C, Thomson NC. Smoking and airway inflammation in patients with mild asthma. *Chest* 2001;**120**:1917—22.
31. Dupont LJ, Demedts MG, Verleden GM. Prospective evaluation of the validity of exhaled nitric oxide for the diagnosis of asthma. *Chest* 2003;**123**:751—6.
32. Juniper EF, Bousquet J, Abetz L, Bateman ED. Identifying 'well-controlled' and 'not well-controlled' asthma using the Asthma Control Questionnaire. *Respir Med* 2006;**100**: 616—21.
33. Rhee H, Belyea MJ, Elward KS. Patterns of asthma control perception in adolescents: associations with psychosocial functioning. *J Asthma* 2008;**45**:600—6.
34. Bender BG. Depression symptoms and substance abuse in adolescents with asthma. *Ann Allergy Asthma Immunol* 2007; **99**:319—24.
35. Bender BG. Risk taking, depression, adherence, and symptom control in adolescents and young adults with asthma. *Am J Respir Crit Care Med* 2006;**173**:953—7.