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Exhaled nitric oxide as a marker of asthma control in smoking patients

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Abstract

Fractionated exhaled nitric oxide (*F*eNO) which is a reliable marker of eosinophilic airway inflammation, is partially suppressed by tobacco smoking. Consequently, its potential as a biomarker in asthma management has never been evaluated in smoking patients. In the present study, we tested the validity of *F*eNO to predict asthma control in this population.

To do this, *F*eNO and the Asthma Control Questionnaire (ACQ) were recorded at least once in 411 non-smoking (345 with at least two visits) and 59 smoking (51 with at least two visits) asthma patients.

Despite similar mean ACQ scores (1.5 vs1.7-p>0.1) *F*eNO was reduced in smoking asthmatics (18,1 ppb vs 33,7 ppb; p<0.001). A decrease in *F*eNO < 20% precludes asthma control improvement in non-smoking (NPV 78%) and in smoking patients (NPV 72%). An increase in *F*eNO <30% is unlikely to be associated with deterioration in asthma control in both groups of patients (NPV= 86% and 84%).

It is concluded that, even in smokers, sequential changes in *F*eNO have a relationship to asthma control. This is the first study indicating that cigarette smoking does not obviate the clinical value of measuring *F*eNO in asthma among smokers.

INTRODUCTION

Although the debate is not over, it is generally accepted that fractionated exhaled nitric oxide (*F*eNO) has the potential to be useful in the management of asthma [1-6]. However, several factors confounding *F*eNO measurement have been recognized [7]. Among them, tobacco smoking has been consistently shown to reduce *F*eNO levels [6,8-15], by a factor varying from 0.63 to 0.80 according to the multivariate analyses that have compared *F*eNO in smokers and in non smokers [6,14,15]. The mechanism by which smoking causes *F*eNO reduction is not fully understood but may include reduction in NO synthesis due to feedback inhibition induced by high concentrations of NO contained in cigarette smoke [9]. NO oxidation or interaction with other molecules present in tobacco smoke might also occur [16]. However, regardless of the mechanism of *F*eNO reduction reported in smokers, it is generally assumed that *F*eNO should not be assessed in asthmatic patients who smoke. Perhaps, consequently, this population (approximately 25 % of adult asthma patients [17]) has been excluded from clinical trials that have explored the potential of *F*eNO as a biomarker in asthma management. Even in our own studies where we have recently shown that *F*eNO is a reliable marker of asthma control over time in unselected patients, but once again, smoking patients were not enrolled [18]. Interestingly, this study strongly suggested that it is the change in *F*eNO values, rather than absolute cut-off points (i.e. individualized *F*eNO profiles), which may be meaningful for the longitudinal assessment of asthma control in daily practise. Therefore, in the present study, we investigated whether, despite the *F*eNO reduction reported in smoking asthma patients [6,8-15], changes in *F*eNO might be significantly related to changes in asthma control in this population as well.

To do this, *F*eNO was monitored on several occasions in smoking and non smoking patients attending a tertiary asthma clinic. Its ability to reflect improvement or worsening of asthma control over time was compared in both groups, using the Asthma Control Questionnaire (ACQ) [19] as a gold standard for the assessment of asthma control

METHODS

Subjects

Between January 1, 2004 and July 30, 2008, 411 adult non smokers and 59 adult smokers attending the Allergy and Asthma clinic in the Chest Department of Erasme University Hospital for treatment of persistent asthma diagnosed according to standard criteria [20] were enrolled in the study.

The study was approved by the local ethics committee and patients signed an informed consent.

Study procedures and design

Study design

The study is a post hoc analysis of an existing database that is continuously updated. A significant part of the current database was reported in our previous publication that documented a relationship between asthma control and *F*eNO in non smoking asthma patients [18]. In the present analysis, we focus on the question as whether current smoking annuls the validity of *F*eNO measurements to predict asthma control. ACQ scores and *F*eNO were recorded independently on one or more occasions for each patient, including smokers who were excluded from the initial analysis for reasons mentioned earlier. At each visit, asthma treatment was adjusted according to GINA guidelines recommendations [20], regardless of ACQ score or *F*eNO value, which were recorded separately.

Since optimal asthma control appears more difficult to achieve in smoking patients [21], the 1.5 optimum cut-off point identifying poorly controlled asthma [22] was selected as the reference ACQ score in the ROC curve analysis. For clarity's sake, we considered that an ACQ score < 1.5 identified asthma that is controlled (i.e. partly or well controlled) whereas an ACQ score ≥ 1.5 identified uncontrolled asthma. Using the ROC curve analysis, we assessed

the ability of *F*eNO to: (i) reflect asthma control cross-sectionally using an ACQ threshold of 1.5 , (ii) detect a significant improvement or worsening of asthma control that resulted in a change from uncontrolled (ACQ≥1.5) to controlled (ACQ< 1.5) asthma respectively or vice versa and (iii) detect a significant improvement or worsening of asthma control defined as a decrease or increase in ACQ of 0.5 or greater even though it was not large enough to result in a change of the asthma control status

Patients treated with low (≤500 µg BDP eq.day^{-1}) and high-to-moderate (>500 µg BDP eq.day^{-1}) ICS doses were considered separately. Indeed, in our previous study [18], we found that the overall ability of *F*eNO to reflect asthma control was reduced in patients using high ICS doses.

Study procedures

(a) Asthma Control Questionnaire (ACQ)

Asthma control was assessed using a French translation of the short version of the Asthma Control Questionnaire (ACQ) from Juniper *et al* [23]. This version does not include FEV_1 rating**.** Patients subjectively evaluate the degree of impairment caused by their asthma during the preceding seven days by responding to six questions using a 7-point scale—a score of 0 indicates no impairment and a score of 6 indicates maximal impairment. The total ACQ score is the mean of the six responses, varying therefore between 0 (totally controlled asthma) and 6 (totally uncontrolled asthma). A score of greater than 1.5 is used to identify poorly controlled asthma [22]. A 0.5 change in the ACQ score is considered to be the minimum change that is clinically relevant [22].

(b) Fractionated Exhaled nitric oxide (*F*eNO)

*F*eNO was measured before any forced expiratory manoeuvres using a daily calibrated LR 2000 chemo-luminescence analyser (Logan Research LTD, Rochester, UK) with on-line measurement of a single exhalation at flow rate of 50ml/s (ATS/ERS standard) [24]. *F*eNO levels were read at the plateau corresponding to 70-80% of the CO_2 curve. Absolute *F*eNO values are expressed in ppb, and changes in *F*eNO are expressed as a percentage of the initial value (Δ %).

Statistical methods

ROC curve analysis was performed in the whole population as well as in different sub-groups: patients treated by low and high-to-moderate doses. The area under the ROC curve (AUC) was computed and its difference from 0.5 was statistically evaluated (MedCalc®). For a given type of assessment, the optimal cut-off value was determined for the whole population by maximizing the Youden's index [25], i.e. the true positive rate (sensitivity) minus the false positive rate (1-specificity) (see supplementary on-line material). Geometrically, this index is the vertical distance between the ROC curve and the first bisector. The cut-off value corresponding to the maximum value of Youden's index was then used to derive sensitivity, specificity, positive and negative predictive values, and accuracy in the whole population and in the sub-groups of patients. In the supplementary on-line material, Se, Sp, PPV, NPV and accuracy may be found for other cut-off values, as well as the amounts of true positive-, true negative-, false positive- and false negative cases (contingency tables).

Unpaired t-tests were used when considering FEV_1 and log-transformed *F*eNO values and Mann-Whitney U tests when considering ICS doses and ACQ scores. Proportions were compared using a $\chi 2$-test.

The limit of significance is 0.05.

RESULTS

Three hundred forty-five of 411 non-smoking patients and 51 of 59 smoking patients were seen at least twice, representing 646 and 92 pairs of successive visits for non-smoking and smoking patients, respectively (median time between two visits: non-smoking patients: 88 days, range 10 – 1255 days, inter-quartile interval 42 -189; smoking patients: 93 days, range 7 - 525 days, inter-quartile interval 49 -182). Table 1 presents, in the non-smoking and in the smoking group, demographic data as well as *F*eNO, FEV_1, ACQ score and ICS dose values at study onset for the total studied population (included in the cross-sectional analysis) and in the sub-group of patients who were seen at least twice (included in the longitudinal analysis). Tables 2-5 display the cut-off values (resulting from Youden's index maximisation), the number of positive and total cases, and therefore the prevalence, the sensitivity (Se), the specificity (Sp), the positive (PPV) and negative (NPV) predictive values, the accuracy and the p value allowing to reject (or not) the null hypothesis AUC=0.5.

Cross-sectional assessment of asthma control

Asthma control was cross-sectionally assessed at study onset for 411 non-smoking and 59 smoking asthma patients. Controlled asthma (ACQ score < 1.5) was considered as a positive event. Table 2 shows that, in smoking asthma patients, *F*eNO is unable to cross-sectionally assess asthma control.

Assessment of change in asthma control between pairs of visits

Change from uncontrolled (ACQ score ≥ 1.5) to controlled (ACQ score < 1.5) asthma

In non-smoking and smoking patients, asthma was uncontrolled at visit 1 in 283 pairs (out of 646) and 52 pairs (out of 92), respectively. A change to controlled asthma (spontaneous as

well as treatment induced) at visit 2 is considered as a positive event. This was observed in 133 and 17 occasions, in non-smoking and smoking patients, respectively.

Table 3 shows that *F*eNO exhibits high operating characteristics in both non-smoking and smoking groups. The cut-off values for decrease in *F*eNO which had the highest negative predictive values for establishing control were 30% in non-smokers and 20% in smokers.

Change from controlled (ACQ score < 1.5) to uncontrolled (ACQ score ≥ 1.5) asthma

In non-smoking and smoking patients, asthma was controlled at visit 1 in 360 pairs (out of 643) and 40 pairs (out of 92), respectively. A change to uncontrolled asthma at visit 2 is considered as a positive event. This was observed on 65 and 10 occasions, in non-smoking and smoking patients, respectively.

Table 4 shows that *F*eNO exhibits high operating characteristics in both non-smoking and smoking groups. The cut-off values for increase in *F*eNO which had the highest negative predictive values for a change to uncontrolled asthma control was 50% in both non-smokers smokers.

Improvement of asthma control (ΔACQ < -0.5)

A significant improvement of asthma control between two consecutive visits is considered as a positive event. As a whole, this occurred in 257 and 40 occasions, in non-smoking and smoking patients, respectively.

Table 5 shows that, in the entire population, *F*eNO exhibited similar operating characteristics in non-smoking and smoking patients. Figure 1 illustrates this feature.

When considering the sub-group of smoking patients treated with more than 500 μg eq.BDP.day^{-1}, *F*eNO is no longer significant in assessing an improvement of asthma control.

Worsening of asthma control (ΔACQ > 0.5)

A significant worsening of asthma control between two consecutive visits is considered as a positive event. As a whole, this occurred in 161 and in 26 occasions, for non-smoking and smoking patients, respectively.

Table 6 shows that, as for improvement assessment, *F*eNO exhibited analogous operating characteristics in non-smoking and smoking patients. With a cut-off value at 30% change, a high negative predictive value is observed in both groups.

When considering the sub-group of smoking patients treated with more than 500 μg eq.BDP.day^{-1}, *F*eNO operating characteristics in assessing asthma control worsening is less significant.

In both improvement (Table 5) and worsening (Table 6) assessment of asthma control, we considered a sub-group of pairs of visits with an initial ACQ score <2 as well as a sub-group of pairs of visits without ICS dose modification. Overall, *F*eNO characteristics are only mildly affected compared to the total group.

DISCUSSION

The present study confirms that, compared to non-smokers, *F*eNO is reduced in smoking asthma patients. However, this reduction does not appear to suppress its ability to reflect asthma control in smoking patients, as long as changes in *F*eNO values detected by repeated measurements are considered.

*F*eNO is a reliable marker of eosinophilic airway inflammation [26] that has the potential to be useful in the management of asthma [1-5]. However, tobacco smoking, which affects ±25% of the asthma population [17], leads to a decrease in *F*eNO [6,8-15] and is considered as a confounding factor. Therefore, it is generally assumed that *F*eNO should not be assessed in asthmatic patients who smoke.

At first glance, our results seem to support this common paradigm. *F*eNO levels were in fact substantially reduced in smoking as compared to non-smoking asthma patients and to an extent that is similar to that found in previous studies [6,14,15]. Furthermore, whereas a single *F*eNO value was confirmed to be significantly related to asthma control in the non-smoking population (i.e. *F*eNO level > 50ppb indicates uncontrolled asthma in most cases) [18], such a relation could not be found in the smoking population.

However, in our own previous study [18] which involved non-smoking patients, we showed sequential *F*eNO assessments to be more useful than isolated measurements in demonstrating asthma control. In our current study, we found this to also hold true for smoking asthma patients. Indeed, repeated *F*eNO measurements do appear helpful with regard to indicating change in asthma control over time in both populations. Thus, when asthma is uncontrolled in non-smoking patients, a *F*eNO reduction by at least 30% would predict that asthma is controlled in two out of three cases. The degree of change in *F*eNO one should be concerned with for smoking patients is different: a *F*eNO reduction < 20% would indicate that asthma remains uncontrolled in most cases. Conversely, when asthma is controlled, a *F*eNO increase <50% would indicate that asthma remains controlled in either population.

The aim of asthma treatment is to achieve full asthma control (ACQ< 0.75). In smoking patients, however, optimal control is usually more difficult to achieve [21,27,28], most likely due to the reduction in anti-asthma drugs effectiveness that was recently documented in this

population [27,28]. The present study confirms this: well-controlled asthma (ACQ score< 0.75) was achieved in only 15 % of smoking patients compared to 33% in non-smokers ($p<0.001$-data not shown). Treatment adjustments resulted in asthma that could no longer be considered poorly controlled in as much as 33% of smoking patients (data not shown). For this reason, we felt an ACQ cut-off score of 1.5 (which identifies poorly controlled asthma), to be more appropriate for the present data analysis, and selected it for the current study. Since this level of control was achieved in only 33% of our patients, we also considered the ability of *F*eNO to detect any significant improvement in asthma control [22]. In this respect, repeated *F*eNO assessments appear again helpful in both populations: a *F*eNO reduction <20% indicates in most cases that no significant improvement in asthma control has occurred. Conversely, *F*eNO increases <30% are helpful to rule out mild deteriorations of asthma control. The results may be summarized as: if *F*eNO does not change as indicated, the level of asthma control is not modified. This seems to remain true whether the initial ACQ score is high or low and whether ICS dose was modified or not.

Interestingly, when patients were treated with high-to-medium ICS doses, *F*eNO no longer had the ability to reflect an improvement in asthma control for smoking patients, whereas for non-smoking patients its ability was only slightly reduced. A similar trend is observed with respect to asthma control deteriorations. These results confirm the overall reduction of *F*eNO ability to reflect asthma control in patients treated with high-to-medium ICS doses that we documented in our previous study [18]. In addition, it appears that confounding factors such as high ICS doses [29,30] and tobacco smoking [6,8-15] which are known to reduce *F*eNO would have a cumulative interfering effect that may eventually suppress *F*eNO ability to reflect asthma control. This suggests that the effect of these confounding factors might have to be taken into account when using *F*eNO to assess asthma control. This needs to be clarified by appropriately designed studies.

In conclusion, this is the first study indicating that cigarette smoking does not obviate the clinical value of measuring *F*eNO in asthma. Indeed, it is shown that even in smokers,

sequential changes in *F*eNO have a relationship to asthma control. The results also suggest that factors such as smoking and ICS dose act cumulatively to influence the ability of *F*eNO to be used to assess asthma control. Overall, the importance of sequential *F*eNO measurements in smokers or non-smokers is to distinguish whether or not ongoing or a change in respiratory symptoms is due to changes in airway inflammation - possibly requiring a change in anti-inflammatory therapy. Our data provide evidence which enables the magnitude of changes in *F*eNO to be more accurately interpreted when addressing this important question

Table 1: Demographic data and indices values at study onset

	Total			Patients seen at least twice		
	Non-smokers	**Smokers**	**p[&]**	**Non-smokers**	**Smokers**	**p[&]**
N	411	59		345	51	
Age[§]	41 ± 16	38 ± 11	0.39	41 ± 16	39 ± 11	0.47
M/F	195/216	34/25	0.14	219/126	26/25	0.08
Non-atopic/Atopic	61/350	5/54	0.19	43/302	4/47	0.34
ACQ score[*]	1.5 [0-5.0]	1.7 [0-5.3]	0.34	1.7 [0-5.3]	1.9 [0-5.3]	0.34
ICS dose[*$]	250 [0 – 2000]	500 [0 – 2000]	0.50	250 [0 – 2000]	500 [0 – 2000]	0.37
***F*eNO (ppb)[#]**	33.7 [14.3 – 79.2]	18.1 [6.9 – 47.5]	<0.001	34.8 [14.6 – 83.0]	18.5 [6.1 – 55.5]	<0.001
FEV_1 (%pred)[§]	85.6 ± 15.7	86.2 ± 17.9	0.80	85.0 ± 20.6	86.5 ± 18.0	0.79

Data are presented as: [#]: geometrical mean [geometrical interval] [§] : mean±SD; [*] median [range]; [$] : ICS dose in µg equ BDP.day^{-1}; [&] : comparison between non-smoking and smoking group. N is the number of patients in each group. Except for *F*eNO, non-smoking and smoking groups are statistically similar.

Table 2: Cross-sectional assessment of asthma control.

	N	n^+	P (%)	Cut-off (ppb)	Se (%)	Sp (%)	PPV (%)	NPV (%)	Acc (%)	p
Non-smokers	411	197	48	50	72	56	61	68	64	<0.001
Smokers	59	15	25	25	66	48	30	81	53	0.39

Data are presented as: N, n+ and P are the total number of events, the number of positive cases, and the prevalence, respectively. Se, Sp, PPV, NPV and p are sensitivity, specificity, positive and negative predictive values, and the statistical significance of rejecting AUC=0.5, respectively. A positive event is controlled asthma (ACQ score < 1.5). A true positive case is defined as *F*eNO≤cut-off value associated with a controlled asthma. *F*eNO does not discriminate cross-sectionally controlled versus uncontrolled asthma in smoking patients (p=0.39).

Table 3: Assessment of a change from uncontrolled (ACQ score ≥ 1.5) to controlled (ACQ score < 1.5) asthma

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	N	n^+	P (%)	Cut-off (%)	Se (%)	Sp (%)	PPV (%)	NPV (%)	Acc (%)	p
Non-smokers	283	133	47	-30	68	71	68	72	70	<0.001
Smokers	52	17	33	-20	71	66	50	82	67	0.016

Data are presented as: N, n+ and P are the total amount of events, the amount of positive cases, and the prevalence, respectively. Se, Sp, PPV, NPV, Acc and p are sensitivity, specificity, positive and negative predictive values, accuracy and the statistical significance of rejecting AUC=0.5, respectively. A positive event is a change from uncontrolled (ACQ score ≥ 1.5) to controlled (ACQ score < 1.5) asthma. A true positive case is defined as *F*eNO change≤cut-off value (e.g. -40%) associated with a positive event. *F*eNO exhibits similar operating characteristics in both non-smoking and smoking group. Particularly, a high NPV is observed.

Table 4: Assessment of a change from controlled (ACQ score < 1.5) to uncontrolled (ACQ score ≥ 1.5) asthma

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	N	n^+	P (%)	Cut-off (%)	Se (%)	Sp (%)	PPV (%)	NPV (%)	Acc (%)	p
Non-smokers	360	65	18	+50	42	75	26	86	69	0.001
Smokers	40	10	25	+50	68	87	63	89	83	0.017

Data are presented as: N, n+ and P are the total amount of events, the amount of positive cases, and the prevalence, respectively. Se, Sp, PPV, NPV, Acc and p are sensitivity, specificity, positive and negative predictive values, accuracy and the statistical significance of rejecting AUC=0.5, respectively. A positive event is a change from controlled (ACQ score < 1.5) to uncontrolled (ACQ score ≥ 1.5) asthma. A true positive case is defined as *F*eNO change≥cut-off value associated with a positive event. *F*eNO exhibits similar operating characteristics in both non-smoking and smoking group. Particularly, a high NPV is observed.

Table 5: Assessment of asthma control improvement (ΔACQ < -0.5)

		N	n^+	P (%)	Se (%)	Sp (%)	PPV (%)	NPV (%)	Acc	p
Non-smokers (-20%)[&]	**Total**	643	257	40	64	71	61	74	68	<0.001
	D ≤ 500[§]	306	116	38	74	67	58	80	70	<0.001
	D > 500[§]	337	141	42	55	74	60	70	66	<0.001
	ACQ<2	432	112	26	60	70	41	83	66	<0.001
	ΔD = 0	301	108	36	53	66	47	72	65	0.002
Smokers (-20%)[&]	**Total**	92	40	43	57	74	62	70	66	<0.001
	D ≤ 500[§]	35	14	41	62	84	75	78	77	<0.001
	D > 500[§]	57	26	46	50	71	59	63	61	0.070
	ACQ<2	53	18	34	56	77	56	77	70	<0.001
	ΔD = 0	47	15	32	67	75	57	83	72	<0.001

Data are presented as: [§] : ICS dose (D) in µg equ BDP.day^{-1}; [&] : cut-off value. ACQ<2 row tests the subgroup with an initial ACQ score < 2 and ΔD=0 row tests the sub-group without treatment modification between consecutive visits. N, n+ and P are the total amount of events, the amount of positive events, and the prevalence, respectively. Se, Sp, PPV, NPV, Acc and p are sensitivity, specificity, positive and negative predictive values, accuracy and the statistical significance of rejecting AUC=0.5, respectively. A positive event is defined as an improvement in asthma control. A true positive case is defined as a *F*eNO change ≤ cut-off value (e.g. -25%) associated with an improvement of asthma control between consecutive visits. When smoking patients are treated with high ICS dose, *F*eNO loses its ability to assess a control improvement (p=0.07).

Table 6: assessment of asthma control worsening (ΔACQ > +0.5)

		N	n^+	P (%)	Se (%)	Sp (%)	PPV (%)	NPV (%)	Acc (%)	p
Non-smokers (+30%)[&]	**Total**	643	161	25	51	76	37	84	70	<0.001
	D ≤ 500[§]	306	64	21	67	76	43	90	74	<0.001
	D > 500[§]	337	97	29	42	78	44	77	68	<0.001
	ACQ<2	432	130	30	54	73	47	79	67	<0.001
	ΔD = 0	301	99	33	48	70	34	80	65	<0.001
Smokers (+30%)[&]	**Total**	92	26	28	67	77	52	86	74	<0.001
	D ≤ 500[§]	35	11	31	70	91	78	87	86	<0.001
	D > 500[§]	57	15	26	64	71	43	85	70	0.037
	ACQ<2	53	17	32	71	86	71	86	81	<0.001
	ΔD = 0	47	14	30	57	73	47	80	68	0.025

Data are presented as: [§] : ICS dose (D) in µg equ BDP.day^{-1}; [&] : cut-off value. ACQ<2 row tests the subgroup with an initial ACQ score < 2 and ΔD=0 row tests the sub-group without treatment modification between consecutive visits. N, n+ and P are the total amount of events, the amount of positive events, and the prevalence, respectively. Se, Sp, PPV, NPV, Acc and p are sensitivity, specificity, positive and negative predictive values, accuracy and the statistical significance of rejecting AUC=0.5, respectively. A positive event is defined as a worsening of asthma control. A true positive case is defined as a *F*eNO change ≥ cut-off value associated with a worsening of asthma control between consecutive visits. When smoking patients are treated with high ICS, *F*eNO ability to detect a worsening of control is somewhat reduced (p=0.037).

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Legend to the figure

Figure 1: ROC curve characterizing the ability of *F*eNO to assess an improvement of asthma control defined as a significant ACQ score decrease (Δ ACQ score > 0.5) between two consecutive visits. The solid line and the dashed lines represent non-smoking and smoking patients, respectively. *F*eNO exhibits similar operating characteristics in both populations.

Figure 1