Airway calibre variation is a major determinant of exhaled nitric oxide's ability to capture asthma control

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Clinicians must take into account FEV1 changes when using *F*ENO as a marker of asthma control
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Cite this article as: Michils A, Haccuria A, Michiels S, *et al*. Airway calibre variation is a major determinant of exhaled nitric oxide's ability to capture asthma control. *Eur Respir J* 2017; 50: 1700392 [https://doi.org/10.1183/13993003.00392-2017].

ABSTRACT Changes in airway calibre have the potential to modify exhaled nitric oxide fraction (*F*ENO) values and could hamper how *F*ENO captures changes in asthma control. Here, our objective was to assess whether forced expiratory volume in 1 s (FEV1) variations alter the ability of *F*ENO to reflect asthma control.

*F*ENO, asthma control (Asthma Control Questionnaire (ACQ)) and FEV1 were measured at least two times in 527 patients during 1819 pairs of visits. Determinants of *F*ENO–ACQ discordance probability were evaluated through a logistic regression analysis. The effectiveness of *F*ENO at capturing either asthma control worsening or improvement between two visits was then assessed by undertaking a stratified receiver operating characteristic curves analysis.

When FEV1 and *F*ENO change in the same direction, the odds of *F*ENO–ACQ being discordant are multiplied by 3 ($p<0.001$). The area under the curve values were 0.765 (95% CI 0.713–0.805) (improvement; $p<0.001$) and 0.769 (95% 0.706–0.810) (worsening; $p<0.001$) or 0.590 (95% 0.531–0.653) (improvement; $p=0.001$) and 0.498 (95% 0.416–0.567) (worsening; $p=0.482$) when FEV1 and *F*ENO changed in the opposite or same direction, respectively.

The manner in which *F*ENO and FEV1 vary concomitantly when asthma control changes determines the ability of *F*ENO to capture this change: parallel or opposite changes in FEV1 and *F*ENO either decrease or increase this ability to capture asthma control changes.

This article has supplementary material available from erj.ersjournals.com

Received: Dec 09 2016 | Accepted after revision: May 17 2017

Support statement: AstraZeneca provided a grant for the Immunobiology of Asthma Unit. Funding information for this article has been deposited with the Crossref Funder Registry.

Conflict of interest: Disclosures can be found alongside this article at erj.ersjournals.com

Introduction

Current guidelines indicate that the exhaled nitric oxide fraction (F_{ENO}) of asthma patients marks T-helper cell type 2 (Th2-type) airway inflammation [1] and holds potential application in asthma management [1–3]. Asthma control is a major goal of asthma management [4]. An initial longitudinal study by Jones *et al.* [5] showed the usefulness of F_{ENO} monitoring for predicting and diagnosing loss of asthma control. However, the ability of F_{ENO} to detect deteriorating asthma was shown to be rather limited in other controlled trials using reduction or short withdrawal of inhaled corticosteroid (ICS) therapy [6, 7]. In a study involving unselected asthma patients, we confirmed that F_{ENO} is a valuable, yet far from perfect, marker of asthma control [8]. The overall ability of F_{ENO} to reflect asthma control change was shown to be particularly reduced in patients suffering from severe asthma and deteriorated lung function. This suggested a possible impact of airway calibre reduction on the ability of F_{ENO} to capture asthma control. Several studies focusing on airway challenges have demonstrated that a reduction of airway calibre reduces F_{ENO} levels in the absence of any inflammatory changes [9–12]. This is most likely due to the decrease of available epithelial surface, which impairs NO diffusion from the airway epithelium into the airway lumen [13, 14]. The involvement of peripheral airways amplifies this reduction because most NO production is concentrated in small conductive airways [15], which represent the vast majority of the total epithelial surface. Moreover, an acute increase of airway calibre was recently shown to also affect F_{ENO} values in a manner depending on the site of bronchodilation (*i.e.* no change, increase and decrease with the relief of central, distal or peripheral airway obstruction, respectively) [16].

Combined, these data indicate that variations of forced expiratory volume in 1 s (FEV_1) interfere with F_{ENO} values and therefore with the ability of F_{ENO} to reflect airway inflammation. In a model using allergen challenge, it was even shown that, during the late inflammatory-phase reaction, the reduction of airway calibre completely counteracted the boosting effect of airway inflammation on F_{ENO} levels (*i.e.* the persistent reduction of FEV_1 completely overriding the increased production of NO usually associated with the amplification of the inflammatory process) [12].

With the above in mind, in the present study we set out to explore whether changes in airway calibre that affect the ability of F_{ENO} to reflect airway inflammation might also interfere with its ability to capture asthma control. To this end, a logistic regression was performed on data from asthmatic patients who attended the asthma outpatient clinic at least two times in order to establish whether airway calibre F_{ENO} interaction significantly impacts the probability for a F_{ENO} change to reflect an asthma control change. When it appeared that this parameter was a potential influencing factor, its impact on the ability of F_{ENO} to capture asthma control was then quantified by means of a stratified receiver operating characteristic (ROC) curves analysis.

Methods

Subjects

Adult patients from the Allergy and Asthma Clinic in the Chest Dept of Erasme University Hospital (Brussels, Belgium) were enrolled in the study between January 1, 2004 and January 1, 2014. Asthma was diagnosed according to standard criteria [4]. As the study was conducted in a standard clinical context, all patients with a definite diagnosis of asthma were considered for inclusion with the exception of smokers. This is because studies showed that F_{ENO} is suppressed by tobacco smoking [17]. Asthma severity was evaluated in the *post hoc* analysis according to recent international recommendations [18].

Patients were asked not to use short-acting β_2-agonists 6 h prior to visiting the clinic.

The local ethics committee approved the study and participating patients signed an informed consent form.

Study design

The present study can be described as a *post hoc* analysis of an existing database that is continuously being updated. The authors reported a significant part of this database in previously published studies, during which they documented the relationship between asthma control and F_{ENO} in asthma patients [8, 19]. The present analysis focuses on what impact airway calibre change has on the ability of F_{ENO} to capture a change in asthma control. For each patient, Asthma Control Questionnaire (ACQ) scores, F_{ENO} and pre-bronchodilator FEV_1 were recorded independently on one or more occasions. During each visit, asthma treatment was adjusted following Global Initiative for Asthma guidelines [4], regardless of ACQ score or F_{ENO} value, which were recorded separately.

Patients were eligible for inclusion in the *post hoc* analysis if they attended the outpatient clinic at least two times.

Study procedures

Asthma Control Questionnaire

Asthma control was assessed by means of a French translation of the short version [20] of the ACQ from Juniper *et al.* [21]. This version does not include FEV1 rating. The ACQ score varies between 0 (totally controlled asthma) and 6 (totally uncontrolled asthma). A score <0.75 is used to identify well-controlled asthma [22]. A 0.5 change in the ACQ score is viewed as the minimum change that is clinically relevant [22].

Exhaled nitric oxide fraction

*F*ENO was measured before any forced expiratory manoeuvres using a daily calibrated LR 2000 chemiluminescence analyser (Logan Research, Rochester, UK) with online measurement of a single exhalation at flow rate of 50 $mL \cdot s^{-1}$ (American Thoracic Society (ATS)/European Respiratory Society (ERS) standard) [2]. Exhaled NO levels were read at the plateau corresponding to 70–80% of the CO_2 curve. Absolute *F*ENO values are expressed in parts per billion.

Lung function

Spirometry was performed using a ZAN300 spirometer (nSpire Health, Oberthulba, Germany). Pre-bronchodilator FEV1 was used as an index of airway calibre. FEV1 values are expressed as a percentage of the predicted value [23].

Statistical methods

Among the group of patients who attended the outpatient clinic at least two times, changes in FEV1 (ΔFEV1), *F*ENO (Δ*F*ENO) and ACQ (ΔACQ) were measured between each visit. The ability of *F*ENO change to capture an absolute change of ACQ (|ΔACQ|) $\geqslant$0.5 was evaluated for each pair of visits. A true positive for asthma control improvement is defined as Δ*F*ENO $\leqslant -30\%$ of baseline associated with ΔACQ $\leqslant -0.5$ [8] and for asthma control worsening as Δ*F*ENO $\geqslant$30% of baseline associated with ΔACQ $\geqslant$0.5. Baseline is the value at the first visit of the considered pair of visits.

The total pairs of visits was then randomly divided into two subgroups of comparable size.

In the first subgroup (logistic regression group), a logistic regression was performed to assess what factors influence the probability that Δ*F*ENO accurately assesses the improvement or worsening of asthma control. This regression included as independent variables the change in ICS dose initiated at the first visit, the baseline values of ICS dose, ACQ score, *F*ENO, FEV1, and a binary variable (β) assessing the interaction between Δ*F*ENO and ΔFEV1 for this pair of visits. β=0 if Δ*F*ENO and ΔFEV1 have opposite signs (change in opposite directions); β=1 if they have similar signs (change in identical direction).

In the second subgroup (ROC group), variables appearing to be significant from the logistic regression were used to perform a stratified ROC curves analysis, evaluating the ability of *F*ENO change between the two visits as a percentage of baseline to capture changes in asthma control.

R software (www.r-project.org) was used for statistical analyses. $p<0.05$ was considered statistically significant (two-tailed).

Results

Between January 2004 and January 2014, of the 1039 patients attending the outpatient clinic, 527 came at least two times (281 females/246 males; age (mean±SD) 39±16 years at inclusion). Of these patients, 247 were newly diagnosed and had not received any specific treatment for asthma prior to participating in this study. The remaining 280 regularly attended the outpatient clinic to receive treatment for chronic asthma and were already ICS users with or without other asthma medications, in accordance with recommendations of international guidelines [4]. 448 patients (85%) were found to be allergic after undergoing a skin prick test or radioallergosorbent test against common inhalant allergens.

Prior to any analysis, the degree of autocorrelation of the residuals of the ΔACQ and Δ*F*ENO relationship, ordered according to time, was evaluated by a Durbin–Watson test in every patient who attended the outpatient clinic at least 13 times (12 pairs of visits). Out of 38 patients fulfilling this criterion, the data presented a significant degree of autocorrelation in only three. We therefore considered for analysis all available pairs, culminating in 1819 pairs of visits. The median (interquartile range) interval between two visits was 102 (54–236) days.

Pairs of visits were randomly divided into 923 pairs for the logistic regression group and 896 pairs for the ROC group. Table 1 compares the characteristics of the two groups, which appeared to be similar for first visits as well as for rate of improvement, worsening and β.

TABLE 1 Characteristics of the pairs of visits included in the logistic regression and receiver operating characteristic (ROC) groups

	Logistic regression group	ROC group	p-value[¶]
Pairs of visits	923	896	
ICS dose µg BDPeq·day^{-1}[#]	882±688	860±701	0.391
***F*ENO ppb[#]**	26.5 (11.4–61.7)	26.7 (10.8–66.3)	0.862
FEV1 % pred[#]	78.2±19.3	78.7±19.6	0.427
ACQ[#]	1.8±1.5	1.9±1.5	0.174
Improvement %	37.7	39.7	0.374
Worsening %	24.1	23.5	0.801
β=1 %	41.9	43.1	0.619

Data are presented as n, mean±SD or geometric mean (geometric interval), unless otherwise stated. ICS: inhaled corticosteroid; BDPeq: beclomethasone dipropionate equivalent; *F*ENO: exhaled nitric oxide fraction; FEV1: forced expiratory volume in 1 s; ACQ: Asthma Control Questionnaire. [#]: characteristics at the first visit of the pair; [¶]: p-value evaluated by the unpaired t-test (on log-transformed values for *F*ENO), except for percentages (Chi-squared test).

Logistic regression

Table 2 shows the results of the logistic regression that assessed the impact of the independent variables on the probability for Δ*F*ENO to reflect an asthma control change.

Baseline *F*ENO and β emerged as significant variables. Notably, FEV1 and *F*ENO changing in similar directions (β=1) decrease the odds to capture an asthma control improvement or worsening by 71% and 81%, respectively.

ROC curves analysis

We challenged these results by performing a ROC curves analysis in a separate subgroup (ROC group) in order to evaluate the ability of *F*ENO to capture changes in asthma control.

Table 3 shows area under the curve (AUC) values and their significance for ACQ improvement and worsening assessment, for all pairs of visits (n=896), for pairs corresponding to β=1 and β=0 (a positive or negative Δ*F*ENO/ΔFEV1, respectively) and for pairs corresponding to baseline *F*ENO <25, 25–50 and ⩾50 ppb. Only β=1 yields an important (for improvement assessment) or total (for worsening assessment) loss of the ability of *F*ENO to reflect asthma control (figure 1).

ACQ and FEV1 amplitude changes

We examined FEV1 amplitude changes in subgroups of pairs, exhibiting a strong ACQ change (|ΔACQ| ⩾1, n=740; improvement, n=475; worsening, n=265) or a mild ACQ change (1> |ΔACQ| ⩾0.5, n=398; improvement, n=230; worsening, n=168). A strong ACQ change was associated with a mean±SD ΔFEV1 14.1±30.2% for improvement (p<0.001) and −10.9±16.7% for worsening (p<0.001). A mild ACQ change was associated with mean±SD ΔFEV1 2.8±14.7% for improvement (p=0.005) and −1.8±16.8% for worsening (p=0.162). In the mild ACQ change group, which was associated with trivial FEV1 changes, β was no longer a discriminating factor in a logistic regression (p=0.122 for improvement and p=0.646 for

TABLE 2 Results of logistic regression

	Improvement		Worsening	
	Adjusted OR (95% CI)	**p-value**	**Adjusted OR (95% CI)**	**p-value**
β	0.293 (0.177–0.477)	<0.001	0.386 (0.209–0.7)	<0.001
***F*ENO ppb**	1.026 (1.017–1.036)	<0.001	0.968 (0.951–0.983)	<0.001
ICS 100 µg BDPeq·day^{-1}	1.009 (0.966–1.056)	0.677	0.978 (0.915–1.046)	0.523
ΔICS 100 µg BDPeq·day^{-1}[#]	1.023 (0.986–1.073)	0.26	0.948 (0.88–1.016)	0.142
FEV1 % pred	1 (0.985–1.015)	0.961	0.996 (0.978–1.015)	0.687
ACQ score unit	1.142 (0.956–1.367)	0.145	0.873 (0.662–1.144)	0.327

*F*ENO: exhaled nitric oxide fraction; ICS: inhaled corticosteroid; BDPeq: beclomethasone dipropionate equivalent; FEV1: forced expiratory volume in 1 s; ACQ: Asthma Control Questionnaire. [#]: ICS dose change between visits.

TABLE 3 Area under the curve (AUC) data

	Pairs n	Improvement			Worsening		
		Prevalence %	AUC (95% CI)	p-value#	Prevalence %	AUC (95% CI)	p-value#
Total	896	39.7	0.699 (0.662–0.732)	<0.001	23.5	0.653 (0.609–0.696)	<0.001
β=0	509	42.7	0.765 (0.723–0.805)	<0.001	22.7	0.759 (0.706–0.81)	<0.001
β=1	387	35.8	0.590 (0.531–0.653)	0.001	24.6	0.498 (0.416–0.567)	0.482
***F*ENO ppb**							
⩽25	390	29.1	0.611 (0.545–0.67)	<0.001	27.6	0.648 (0.582–0.708)	<0.001
25–50	263	39.5	0.718 (0.653–0.781)	<0.001	23.6	0.636 (0.558–0.709)	<0.001
>50	243	57.3	0.657 (0.585–0.725)	<0.001	17.0	0.641 (0.542–0.737)	0.003

*F*ENO: exhaled nitric oxide fraction. #: p<0.05 considered significant.

worsening). Conversely, in the strong ACQ change group, which was associated with clinically significant FEV1 changes, β was still very discriminant (p<0.001 for both worsening and improvement).

Intra-individual reproducibility

The frequency of β=1 was examined in the subpopulation of 101 patients who were seen at least six times (median (range) 9 (6–34) visits per patient). Figure 2 shows the number of patients exhibiting a given percentage of pairs associated with β=1. It can be seen that 30% of the patients had infrequent β=1 (<30% of the pairs) and few (9%) had frequent β=1 (>70% of the pairs). The other frequencies of β=1 had relatively similar probabilities.

Discussion

This study shows that when asthma control is modified, the manner in which *F*ENO and FEV1 concomitantly change is a major predictor of the ability of *F*ENO to capture the asthma control variation: parallel or opposite changes in FEV1 and *F*ENO result in either a loss or an improvement of the ability of *F*ENO to capture asthma control changes.

NO measured in the expired air of asthmatic patients is recognised as a valuable marker of Th2-type airway inflammation that could have potential application in asthma management [3]. However, several studies have indicated that variations in FEV1 (*i.e.* decrease or increase) result in immediate *F*ENO changes thus impairing its ability to reflect airway inflammation, especially when peripheral airways are involved [9–12, 16]. This is because the vast majority of NO production is concentrated in small conductive airways [15]. Furthermore, in a model using allergen challenge, it was even shown that during the late

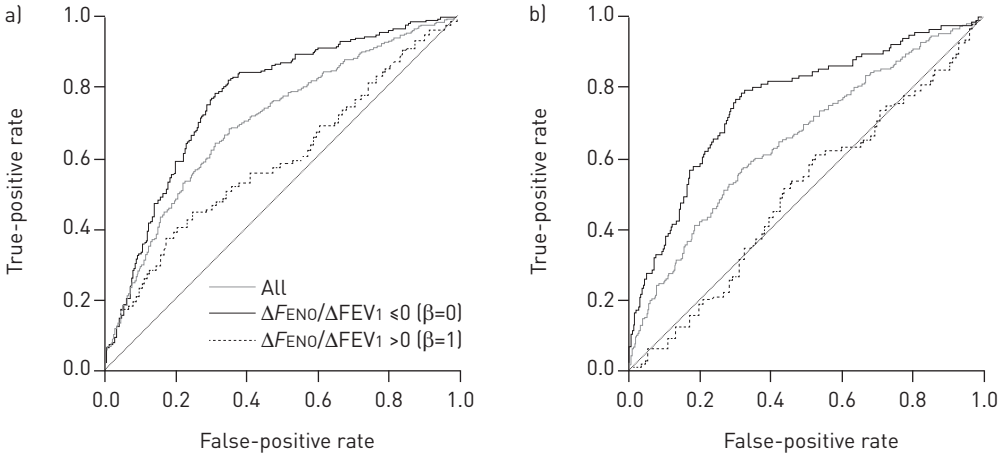

FIGURE 1 Receiver operating characteristic (ROC) curves evaluating the ability of exhaled nitric oxide fraction (*F*ENO) change (as percentage of baseline) to reflect a significant asthma control change: a) improvement or b) worsening. FEV1: forced expiratory volume in 1 s. ROC curves are shown for all pairs of visits, only pairs of visits with Δ*F*ENO/ΔFEV1 ⩽0 (β=0) and only pairs of visits with Δ*F*ENO/ΔFEV1 >0 (β=1).

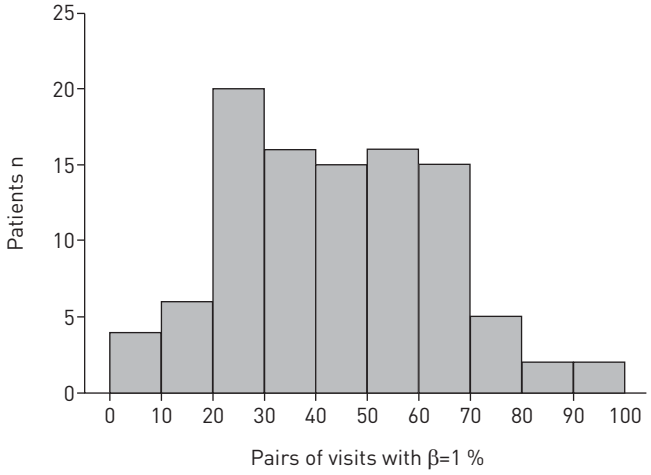

FIGURE 2 Histogram of the number of subjects exhibiting a given percentage of pairs of visits with β=1 (*i.e.* Δ(exhaled nitric oxide fraction)/Δ(forced expiratory volume in 1 s) >0) among at least five consecutive pairs of visits.

inflammatory-phase reaction, the reduction of airway calibre completely counteracted the boosting effect of airway inflammation on *F*ENO levels [12].

This article is proof that variations in airway obstruction may also affect the ability of *F*ENO to reflect asthma control in a complex manner. As shown in figure 1, variations in FEV1 associated with concomitant *F*ENO variations in the same direction (*i.e.* concomitant *F*ENO and FEV1 increases or *vice versa*) nearly or totally diminished the ability of *F*ENO to capture a change in asthma control, whereas FEV1 and *F*ENO changing in opposite directions boosted this ability. To explain this, our proposed hypothesis is based on the main location of the airway obstruction process. FEV1 and *F*ENO changing in the same direction would reflect preferential obstruction up to the peripheral airways, including the small conductive airways where the highest production of NO is concentrated. Consequently, when airway calibre decreases up to this portion of the airways during an asthma attack, the *F*ENO level is reduced even if airway inflammation is increased, as was shown in the allergen challenge experiments, *i.e.* the persistent reduction of airway calibre up to the peripheral airways completely overriding the increased production of NO usually associated with the developing inflammatory process during the late-phase reaction [12]. In stark contrast, FEV1 and *F*ENO changing in opposite directions could reflect a more proximal airway obstruction, involving peripheral airways to a much lesser extent. As a consequence, when an asthma attack associated with airway inflammation occurs, the airway calibre reduction in this zone will not mask the increased NO production that occurs much more peripherally. In a study investigating the acute response to β_2-agonists in asthma patients, using *F*ENO and a single-breath washout test, we demonstrated that the extent of the obstruction process does differ among asthma patients, with some exhibiting airway constriction up to pre-acinar airways, while others display more proximal airway obstruction [16]. The evolution of *F*ENO after β_2-mimetic inhalation differed according to the site of the pre-existing constriction (*i.e.* no change *versus* increase with the relief of proximal *versus* peripheral airway obstruction). Taken together, these findings support the proposed hypothesis. However, this needs confirmation from an appropriately designed prospective trial investigating changes in *F*ENO and FEV1 as well as changes in peripheral obstruction using a single-breath washout test that is taken after the induction of a medically supervised loss of asthma control.

It should be noted that in most cases small changes in ACQ scores ($<\pm1$) were not associated with significant lung function variations. The impact of lung function on the ability of *F*ENO to capture changes in asthma control described in this study therefore only applies to more substantial changes in ACQ scores ($\geqslant\pm1$). Should the asthma worsen, such a relatively large ACQ score increase could potentially be classified as a moderate exacerbation according to ATS/ERS recommendations [24]. Previous studies have already reported a paradoxical parallel increase in FEV1 and *F*ENO during the recovery phase of an acute severe exacerbation [25, 26]. Opposite *F*ENO–FEV1 moves were not reported in those studies that involved relatively small numbers of patients, who experienced severe rather than moderate exacerbation. In addition, the opposite behaviour in FEV1 and *F*ENO change we report when asthma control changes occurs much less frequently (30%) and one cannot deny that this behaviour could be masked in the global mean data presented in those studies.

These results could have implications for clinical practice. It appears that for a given patient seen by the physician, *F*ENO may be most useful as a biomarker of asthma control when changes in asthma control are accompanied by *F*ENO and FEV1 moves in opposite directions compared with values recorded during the

previous visit. Conversely, a new role for *F*ENO may emerge in the management of patients exhibiting FEV1 and *F*ENO changes in the same direction compared with values recorded during the previous visit, when asthma control varies. In this case, physicians should not consider *F*ENO as a reliable biomarker of airway inflammation nor asthma control, but as a marker of peripheral airway involvement in the process, if the aforementioned hypothesis is validated. Approximately a third of frequently attending patients exhibited a repeatedly discordant pattern of *F*ENO and FEV1 movement when their asthma control varied, representing what one may call a "proximal airway obstruction phenotype". This proportion is in line with the number of patients exhibiting no change of *F*ENO after bronchodilation, which was shown to be associated with proximal airway obstruction [16]. For the remaining group of patients, the situation is more complex with few systematic concordant patterns of *F*ENO and FEV1 movement when asthma control varies. This may be explained by the fact that, even if airway calibre and inflammation changes occur in the same distal regions, phase shift and/or time constant differences between the two processes [27] might result in the alternate behaviour patterns that are observed (see supplementary material). Finally, it should be noted that studies that have evaluated how *F*ENO values could help guide ICS treatment in asthmatic patients have not taken into account this lung function parameter, whereas changes in airway calibre frequently occur during the course of asthma [28–31]. This might be the reason behind the somewhat disappointing data available, together with the controversial study designs, selected populations and treatment algorithms already mentioned [32]. These issues require further investigation (*i.e.* taking into account the manner of *F*ENO and FEV1 movement) before any final conclusions can be drawn with respect to *F*ENO use in monitoring asthma control and titrating ICS doses. In all international guidelines on *F*ENO use, lung function should always be recognised as a significant interfering factor in *F*ENO measurements. To date, this has hardly been mentioned.

To conclude, *F*ENO as a biomarker in asthma appears to be profoundly influenced by changes in airway calibre. How *F*ENO can be most useful in asthma management should be identified during each visit on the basis of concomitant FEV1 and *F*ENO movements compared with values recorded during the previous visit when asthma control improves or deteriorates. Opposite *F*ENO and FEV1 changes make *F*ENO a reliable marker of asthma control changes, whereas parallel *F*ENO and FEV1 changes suggest a peripheral airway involvement in the process.

Acknowledgements

The authors thank Mr Tom O'Connell (OCPR, Brussels, Belgium) for proofreading the manuscript.

Author contributions: A. Michils, A. Haccuria and S. Michiels collected data. A. Van Muylem performed the data analysis. A. Michils drafted the manuscript. All authors critically reviewed and approved the final manuscript.

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