Exhaled nitric oxide and asthma control: a longitudinal study in unselected patients

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ABSTRACT

Controlled studies have shown that monitoring exhaled nitric oxide (FENO) improve asthma management. However, they seldom consider the full range of patients seen in clinical practise. In this study, we investigated the ability of FENO to reflect asthma control (AC) over time, and identified, in a regular clinical setting, meaningful FENO cut-points and changes.

AC questionnaire and FENO were recorded at least once in 341 unselected asthma patients. The whole population and sub-groups were considered: inhaled steroïds (ICS) naïve and low or high-to medium (≤ or > 500 µg.equi.BDD.day−1) ICS doses groups.

FENO decrease <40% or increase <30% precludes AC optimization or deterioration respectively (negative predictive value, NPV=79% and 82%). In our low dose group, a decrease >40% indicates AC optimization (positive predictive value, PPV= 83%). In ICS naïve patients, FENO>35ppb predicts AC improvement in response to ICS (PPV=68%). In most cases, FEV1 assessments were not useful.

In conclusion, in a given patient, FENO is significantly related to asthma control over time. The overall ability of FENO to reflect asthma control is reduced in patients using high doses of ICS. FEV1 has little additional value in assessing asthma control.

Word count : 189
INTRODUCTION

Asthma control is a major goal of asthma management [1]. However, asthma is a complex syndrome with several phenotypic elements, such as airway inflammation, airway calibre, bronchial responsiveness, and airway remodelling. Therefore, accurate assessment of asthma control may require a multi-dimensional approach that incorporates distinct parameters, such as symptoms, lung function and biomarkers. Randomised trials have recently shown that asthma management which considered inflammatory markers such as sputum eosinophils and airway hyper responsiveness (AHR) as a surrogate for inflammation resulted in improved asthma control [2, 3]. The degree of airway inflammation may also be reflected by the level of fractionated exhaled nitric oxide (FENO) which is elevated in steroid-naïve asthma [4]. Further increases are seen during asthma exacerbations [5] whereas decreases occur after treatment with ICS [6]. A first longitudinal study by Jones et al. has shown the usefulness of FENO monitoring for predicting and diagnosing loss of asthma control [7]. Furthermore, a one-year follow-up randomised study has caused increased interest in monitoring FENO in asthma patients by demonstrating that FENO-guided asthma therapy resulted in the reduction of ICS doses without compromising asthma control [8]. Although a more recent study may slightly temper this enthusiasm [9], all these data suggest that FENO may be a valuable indicator in the longitudinal assessment of asthma control. However, as with most controlled trials, each of these trials involved only selected patients which do not necessarily represent the full range of clinical situations [10]. In addition, several questions regarding the application of FENO monitoring in the day-to-day management of asthma still remain to be resolved, including the issue of clinically meaningful FENO cut-points and changes [11]. Therefore, we purposely performed a study that documents FENO cut-off values and changes that can be considered clinically important in the longitudinal assessment of asthma control in a population of unselected asthma patients.
To do this, we monitored FENO on several occasions in patients attending a tertiary asthma clinic. Its ability to reflect and to predict improvement or worsening of asthma control over time was evaluated and compared to that of FEV$_1$, using the Asthma Control Questionnaire (ACQ) [12] as a gold standard for the assessment of asthma control.
METHODS

Subjects

Between January 1, 2004 and April 30, 2007, 341 adult patients (164 males- mean age ± SD: 41 ± 16 years) attending the Allergy and Asthma clinic in the Chest Department of Erasme University Hospital for treatment of persistent asthma diagnosed according to standard criteria [1] were prospectively enrolled in the study. One hundred forty two patients were newly diagnosed and had not received any specific treatment for asthma prior to inclusion. The remaining 199 patients regularly attended the out-patient clinic for treatment of chronic asthma and had already being given ICS with or without other asthma medications (long acting beta-2-agonists (LABA) (n= 157), leukotriene antagonists (n= 59), theophylline (n= 27), systemic steroids (n= 16), omalizumab (n=6)…) in accordance with recommendations of the international guidelines [1].

Since the study was conducted in a regular clinical context, all patients with a definite diagnosis of asthma were included with the exception of smokers because it has been shown that FENO is suppressed by tobacco smoking [13]. Furthermore, quite unexpectedly, only 10 percent of our patients were active smokers.

301 patients (88%) were found to be allergic. The allergic status was evaluated using skin prick test or RAST against common inhalant allergens.

Patients were asked not to use β-2-agonists 6 hours prior to visits in the clinic.

The study was approved by the local ethic committee and patients signed an informed consent.
Study procedures and design

Study design

The study was designed as a prospective trial with post hoc data analysis. ACQ scores, FENO and pre-bronchodilator FEV₁ were recorded independently on one or several occasions for each patient. At each visit, asthma treatment was adjusted according to GINA guidelines recommendations, regardless of ACQ score or FENO value, which were recorded separately. Since the ultimate goal of asthma management is to achieve well-controlled asthma, the 0.75 optimum cut point [14] was selected as the reference ACQ score in the ROC curve analysis except when considering severe asthma (see below). Using this technique, we assessed the abilities of FENO and FEV₁ to: (i) reflect asthma control as defined by an ACQ score < 0.75 or >0.75, (ii) detect and predict optimization of asthma control defined as a minimum 0.5 change that allowed the ACQ score to decrease from >0.75 (not well controlled asthma) to <0.75 (well controlled asthma) and (iii) detect and predict loss of optimal control defined as a minimum 0.5 change that allowed the ACQ score to increase from <0.75 (well controlled asthma) to >0.75 (not well controlled asthma).

Patients treated with low (≤500 µg BDP eq.day⁻¹) and high-to-medium (>500 µg BDP eq.day⁻¹) ICS doses were considered separately. Indeed, it has been shown that for ICS doses below 500 µg BDP eq.day⁻¹, the relationship between FENO and the anti-inflammatory effect of ICS is linear while above 500 µg BDP eq.day⁻¹ FENO levels may be low despite ongoing airway inflammation [15-16]. In the latter group (i.e. high-to-medium ICS doses), patients suffering from severe asthma —as defined according the ATS working group criteria [17 ]— were considered separately. In this group, we assessed the FENO ability to detect a significant change in asthma control (i.e. improvement (delta ACQ score>-0.5) rather than optimization (ACQ score< 0.75) and worsening (delta ACQ> +0.5) rather than loss of optimal control).
Indeed, it seemed unreasonable to expect severe asthmatics to achieve the same extent of control as moderate-to-mild asthmatics.

**Study procedures**

(a) *Asthma Control Questionnaire (ACQ)*

Asthma control was assessed using a French translation of the short version [18] of the Asthma Control Questionnaire (ACQ) from Juniper *et al* [12]. 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 (severely uncontrolled asthma). A recent analysis showed that the optimal cut-off point to identify patient whose asthma is well controlled is 0.75 (i.e. if a patient has a score equal or lower than 0.75, there is an 85% chance that his asthma is well controlled) [14]. In addition, a 0.5 change in the ACQ score is considered to be the minimum change that is clinically relevant [14].

(b) *Fractionated Exhaled nitric oxide*

FENO 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) [19]. Exhaled NO levels were read at the plateau corresponding to 70-80% of the CO$_2$ curve. Absolute FENO values are expressed in ppb, and changes in FENO are expressed as a percentage of the initial value (Δ%).
(c) Lung function

Spirometry was performed using a Zan 300 spirometer (Zan®, Oberthulba, Germany). Pre-bronchodilator forced expiratory volume in one second (FEV₁) was used as an index of airway calibre. FEV₁ values are expressed as a percentage of predicted value (% pred) [20], and changes in FEV₁ 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: steroid naïve patients, patients treated by low and high-to-medium doses, severe asthmatics. 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 [21], i.e. the true positive rate (sensitivity) minus the false positive rate (1-specificity). This cut-off value was then used to derive sensitivity, specificity, positive and negative predictive values in the whole population and in the sub-groups of patients.

Unpaired t-tests were used when considering FEV₁ and log-transformed FENO values and Mann-Whitney U tests when considering ICS doses and ACQ scores.

The limit of significance is 0.05.
RESULTS

Table 1 presents FENO, FEV₁, and ACQ score values at study onset for the whole population (n= 341) for ICS naïve patients, for patients treated with low ICS or high-to-medium ICS dose (excluding severe asthmatics) and for patients with severe asthma, respectively.

Two hundred thirty four patients out of 341 were seen at least twice, representing 502 pairs of successive visits (median time between two visits: 80 days, range 10 – 1129 days, inter-quartile interval 42 -180). For non-severe asthmatics, asthma was not well controlled (ACQ score ≥ 0.75) at visit 1 on 251 occasions and well-controlled on 164 occasions. Eighty seven pairs of visits involved patients with severe asthma, which was not well controlled at visit 1 in 73 occasions.

Tables 2-4 display the cut-off values (resulting from Youden's index maximisation, see supplementary on-line material), 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 and the p value allowing to reject (or not) the null hypothesis AUC=0.5. 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). The way to derive PPV and NPV, given Se and Sp, for any given prevalence (Baye's formulas) may also be found in the supplementary material online.

Cross-sectional assessment of asthma control

Asthma control was assessed transversally at study onset for 324 non-severe asthma patients. Well-controlled asthma (ACQ score < 0.75) was considered as a positive event. Table 2 shows in the whole population (n= 324), FENO level > 45ppb or FEV₁<85%pred allows one to exclude a well-controlled asthma (NPV= 89% and 80%, respectively). It must be noted that FENO operating characteristics in asthma control assessment worsen from ICS naïve to
medium-to-high ICS dose group. FEV1 exhibits poor operating characteristics in assessing transversally asthma control.

**Change in asthma control between pairs of visits: assessment and prediction**

**Optimization and improvement assessment**

In non-severe asthma, disease control was not optimal at visit 1 in 251 pairs (out of 415). Optimization of asthma control (spontaneous as well as treatment induced) at visit 2 is considered as a positive event. This occurred in 92 occasions.

Table 3 shows that, with this regards, FENO exhibits good operating characteristics, especially in the low ICS dose group: with a cut-off value at a 40% decrease, a high negative predictive value is observed in all groups of patients. In the group of patients treated with low ICS dose, a high positive predictive (83%) value is also found. Figure 1 illustrates the FENO and FEV1 ROC curves for the total population, patient with low ICS dose, and patients with high-to-medium ICS dose.

Amongst the 73 pairs of visits involving patients with severe and uncontrolled asthma, an improvement in asthma control (i.e. positive event) occurred in 32 occasions. FEV1 and FENO show similar operating characteristics to detect such a change: a FENO decrease lower than 15% or a FEV1 increase less than 5% virtually exclude an improvement in asthma control (NPV = 76% and 74%, respectively) (table 3).

**Loss of optimal control and control worsening: assessment**

In non-severe asthma, an optimal control was documented at visit 1 in 164 pairs (out of 415). Loss of optimal control at visit 2 is considered as a positive event. This occurred in 39 occasions. Table 4 shows that, in the whole population, an FENO increase less than 30% makes a loss of optimal control unlikely (NPV = 82%). Nevertheless, in the high-to-medium
ICS dose group, FENO ability is lost. FEV₁ has poor operating characteristics in all groups of patients, especially in the low ICS dose group.

In severe asthma, a worsening of asthma control (i.e. positive event) occurred in 25 occasions (out of 87 pairs). FEV₁ and FENO exhibit equivalent operating characteristics. A FENO increase lower than 15% or a FEV₁ decrease less than 5% make a worsening of asthma control unlikely (NPV = 78% and 81% respectively).

**Optimization: prediction**

This analysis was restricted to pairs of visits for which asthma was not well controlled initially and for which an anti-inflammatory treatment was started or increased.

In non-severe asthma, 148 pairs of visits fulfilled these conditions and asthma control optimization occurred in 65 occasions (i.e. positive event). The dose increments between the two visits (mean: 523 µg eq. BDP.day⁻¹) were not different in patients who did or did not exhibit asthma control optimization (p =0.39). For those patients already treated with ICS at visit 1, the initial dose (mean: 650 µg eq.BDP.day⁻¹) was similar in the two groups (p = 0.19).

In steroid naïve patients, an initial FENO level higher than 35ppb predicts asthma control optimization in 2 out of 3 cases (PPV= 68%). In ICS treated patients, asthma control is unlikely to become optimal after treatment increase if FENO was lower than 35ppb at visit 1 (NPV = 88%). FEV₁ never predicted optimization. Table 7 in the online data supplement shows the details of the results for several cut-off points.

In the population of severe asthmatics, 27 pairs of visits fulfilled the conditions (asthma non well-controlled at visit 1 and treatment increase) and an improvement was seen in 14 occasions. However, if the anti-inflammatory treatment was similar in patients who did or did not improve their asthma control, the increase in treatment in systemic corticoids was
significantly higher in the group exhibiting an improvement ($p = 0.006$). Given this bias, improvement prediction was not considered in this group of patients

**Loss of optimal control: prediction**

In the population of non severe asthmatics, this analysis was restricted to pairs of visits for which asthma was well controlled initially and for which visits were separated by no more than three months. Sixty one pairs fulfilled these conditions and only 11 pairs exhibited a loss of optimal control at the subsequent visit. No difference appeared with regard to initial ICS dosage nor in treatment modification between patients who did or did not show a loss of optimal asthma control ($p = 0.98$ and $p = 0.61$, respectively). A FENO level lower than 30ppb, along with well-controlled asthma, indicates that a loss of optimal control is unlikely to occur within the next three months (NPV = 94%). FEV$_1$ has no predictive power. Table 8 in the online data supplement shows the details of the results for several cut-off points.

In the group of severe asthmatics, 49 pairs of visits fulfilled the conditions and on only 11 occasions was a worsening of asthma control observed at the subsequent visit. However, if the anti-inflammatory treatment was similar in patients who did or did not worsen their asthma control, a treatment increment in systemic corticoids was significantly higher in the group without worsening ($p = 0.027$). Given this bias, worsening prediction was not considered in the group of severe asthmatics.
DISCUSSION

This study shows that FENO is a reliable marker of asthma control in unselected asthma patients, especially in those patients treated with low doses of ICS. Our data also indicate that changes in FENO values rather than absolute cut-off points (i.e. personalised FENO profiles) may be meaningful for the longitudinal assessment of asthma control.

When we first considered isolated assessment of asthma control (Table 2), we found that a FENO level higher than 45 ppb would indicate that asthma is not well controlled, but only for steroid naïve and low ICS dose treated patients. This is in accordance with the correlation recently documented between FENO and asthma control in newly diagnosed asthmatics, which used another questionnaire (i.e. the Asthma Control Test) — albeit well correlated to the ACQ [14] — to assess asthma control [22]. When considered patients treated with high-to-medium ICS doses, FENO no longer had the ability to reflect asthma control. This may partly explain the discrepancy with recently published data which indicated no association between ACQ score and FENO level in a study in which ICS naïve and ICS treated patients were pooled [23]. In our study, no association was documented between asthma control and single measurement of FEV₁. This confirms previous data showing a poor relationship between airway obstruction and respiratory symptoms in adult asthmatics [24].

These results, taken together, suggest that isolated measurements of FENO and FEV₁ do not appear very successful in capturing asthma control. In fact, control instruments (i.e. questionnaires) may perceive more accurately changes in asthma symptoms rather than asthma symptoms themselves.

However, asthma is a chronic disorder and long-term follow up with repeated assessments of various parameters is required in order to make proper treatment adjustments. Jones et al. [7] were the first to tackle the issue of FENO use in the longitudinal assessment of asthma
control. Using a steroid withdrawal protocol to mimic exacerbations, these authors were able to show that changes in FENO levels (i.e. + 60%) were more useful than single cut points in predicting and diagnosing loss of asthma control. This finding is confirmed in our study involving patients who experienced spontaneous deteriorations of asthma control. In this case, a FENO increase lower than 30% would be helpful to exclude that a significant deterioration of asthma control has occurred (Table 3). The discrepancies in FENO changes documented in the two studies may be explained by differences in the study designs: treatment regimens (i.e. ICS withdrawal vs. ICS maintenance) and study end-points (loss of control vs. loss of optimal control) were different. The difference between the positive predictive values exhibited by FENO in diagnosing loss of control in the two studies (87% in Jones et al. study vs 33% in the present study) is, at least partially, related to the differences in the prevalence of loss of control in each study (78% in Jones et al. study vs. 24% in the present study). It must be noted, finally, that the ability of FENO to detect deteriorating asthma was shown to be rather limited in other controlled trials using reduction or short withdrawal of ICS therapy [25, 26]. In addition, our study indicates that sequential FENO measurements may be also helpful with regard to indicating improvement in asthma control over time. So, when asthma is not optimally controlled, a 40% FENO reduction is a reliable predictor of asthma control optimization, particularly for those patients treated with low ICS doses (PPV 80%). In the group of patients treated with ICS doses higher than 500 µg BDP eq.day⁻¹(almost always ≥1000 µg.day⁻¹ BDP eq in the present study), the ability of FENO to reflect changes in asthma control was somewhat reduced; suggesting that ICS doses might have to be taken into account when using FENO to assess asthma control. However, even in severe asthmatics treated with high-to-very high ICS doses, a 15% FENO change would still apparently be helpful to rule out a significant change in asthma control confirming the usefulness of FENO assessment in this population [27].
Together our data suggest that personalised FENO profiles may be meaningful for asthma follow-up in unselected patients at least when considering asthma control as assessed by the ACQ over a three month period (median time between two visits in the present study). However, it must be acknowledged, that, due to small group sizes, the study was unable to account for factors such as smoking and atopy that may effect on the relationship between FENO and asthma control. So far, it is not known whether the use of FENO profiles rather than absolute cut-offs would have provided different results in the two controlled trials that have investigated the impact of FENO guided therapy on asthma control over one year (8,9). In addition, FENO assessment also appears helpful to predict ICS responsiveness (Table 5). Established guidelines recommend the use of ICS as the first line treatment for chronic asthma [1]. These recommendations are based on clinical studies showing the overall efficacy of ICS for treating asthma. However, heterogeneity in the response to ICS treatment has been reported [28]. In a randomised trial [29], Smith et al have previously documented a 47ppb threshold value that proved to be a reliable predictor of ICS responsiveness in patients with undiagnosed respiratory symptoms. In our study, involving patients with a definite diagnosis of asthma and focusing only on asthma control rather than on the various outcomes investigated in Smith et al. study, a 35ppb threshold value emerges as an helpful predictor of ICS responsiveness: two out of three steroid naïve patients with a FENO level greater than 35ppb have improved control over their asthma after starting a treatment that includes ICS. In those patients who were already treated but not yet optimally controlled, an improvement in asthma control resulting from an increase in ICS dose is much less likely to occur if the FENO level is not greater than 35ppb. Finally, a FENO level lower than 30ppb in stable treated patients predicts that an exacerbation is unlikely to occur within the next three months. The cut-off point is similar to that
documented in a recent controlled trial involving a much more longer follow-up period (i.e. 18 months) [30]

When lung function was considered, FEV1 values did not appear to adequately reflect improvement or worsening of asthma control in the group of patients treated by low ICS doses, who exhibited near normal lung function. This did not hold completely true anymore for more severe asthmatics treated by high ICS doses; these patients had a more altered lung function that left more room for improvement. One might conclude that the lack of control in steroid naïve and low ICS dose treated patients is likely to depend on airway inflammation while in more severe asthmatics treated with high ICS dose it may be the impairment in airway calibre that is the main culprit of poor control.

In conclusion, in a given patient, FENO is significantly related to asthma control over time. The overall ability of FENO to reflect asthma control is reduced in patients using high doses of ICS. FEV1 has little additional value in assessing asthma control.
ACKNOWLEDGMENTS

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REFERENCES


fluticasone and montelukast in childhood asthma. J Allergy Clin Immunol 2005; 115: 233-242


Table 1: FENO, FEV$_1$, and ACQ score values at study onset

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>FENO (ppb)$^#$</th>
<th>p$^&amp;$</th>
<th>FEV$_1$ (%pred)$^§$</th>
<th>p$^&amp;$</th>
<th>ACQ score$^*$</th>
<th>p$^&amp;$</th>
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<tr>
<td>Total</td>
<td>341</td>
<td>32.9 [13-8 – 78.1]</td>
<td></td>
<td>86.3 ± 18.5</td>
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<td>1.5 [0-5.2]</td>
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<tr>
<td>ICS naïve</td>
<td>142</td>
<td>49.8 [24.0 – 103.5]</td>
<td></td>
<td>88.9 ± 17.1</td>
<td></td>
<td>2.0 [0-5.2]</td>
<td></td>
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<tr>
<td>0&lt;D$^#$≤500</td>
<td>102</td>
<td>27.0 [11.7 – 62.1]</td>
<td>&lt;0.001</td>
<td>90.1 ± 15.1</td>
<td>0.59</td>
<td>0.8 [0-4.8]</td>
<td>&lt;0.001</td>
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<tr>
<td>D$^#$&gt;500</td>
<td>80</td>
<td>20.5 [9.0 – 46.7]</td>
<td>&lt;0.001</td>
<td>84.1 ± 19.0</td>
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<td>1.3 [0-5.2]</td>
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<tr>
<td>Severe asthma</td>
<td>17</td>
<td>31.3 [15.9 – 62.1]</td>
<td>0.014</td>
<td>52.8 ± 11.4</td>
<td>&lt;0.001</td>
<td>3.5 [0.8-4.7]</td>
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Data are presented as $^#$: geometrical mean [geometrical interval] $^§$: mean±SD; $^*$: median [range]; $^*$: ICS dose (D) in µg equ BDP.day$^{-1}$; $^&$: comparison with ICS naïve group. N is the amount of patients.
Table 2: cross-sectional assessment of asthma control.

<table>
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<tr>
<th></th>
<th>N</th>
<th>n⁺</th>
<th>P  (%)</th>
<th>Se  (%)</th>
<th>Sp  (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
<th>p</th>
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<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Total</td>
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<td>92</td>
<td>28</td>
<td>83</td>
<td>49</td>
<td>40</td>
<td>88</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>D§ = 0</td>
<td>142</td>
<td>17</td>
<td>12</td>
<td>59</td>
<td>67</td>
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<td>92</td>
<td>0.039</td>
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<tr>
<td>0&lt; D§ ≤ 500</td>
<td>102</td>
<td>46</td>
<td>45</td>
<td>87</td>
<td>34</td>
<td>52</td>
<td>76</td>
<td>0.036</td>
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<td>29</td>
<td>36</td>
<td>93</td>
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<td>40</td>
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<td>Total</td>
<td>324</td>
<td>92</td>
<td>28</td>
<td>74</td>
<td>42</td>
<td>33</td>
<td>80</td>
<td>0.089</td>
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<tr>
<td>D§ = 0</td>
<td>142</td>
<td>17</td>
<td>12</td>
<td>71</td>
<td>38</td>
<td>13</td>
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<td>45</td>
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<td>64</td>
<td>52</td>
<td>44</td>
<td>73</td>
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</table>

Data are presented as $\delta$: ICS dose (D) in µg equ BDP.day$^{-1}$; &: cut-off value. N, n⁺ and P are the total amount of events, the amount 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 a well-controlled asthma. A true positive case is defined as FENO≤45ppb or FEV₁≥85%pred associated with a well-controlled asthma.
Table 3: assessment of an optimization or an improvement of asthma control.

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>n+</th>
<th>P</th>
<th>Se</th>
<th>Sp</th>
<th>PPV</th>
<th>NPV</th>
<th>p</th>
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<tr>
<td><strong>Optimization (non-severe asthma)</strong></td>
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<td></td>
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<tr>
<td>FENO (-40%)</td>
<td>251</td>
<td>92</td>
<td>37</td>
<td>64</td>
<td>78</td>
<td>63</td>
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<td>D ≤ 500§</td>
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<td>56</td>
<td>53</td>
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<td>84</td>
<td>83</td>
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<td>145</td>
<td>36</td>
<td>25</td>
<td>53</td>
<td>75</td>
<td>41</td>
<td>83</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FEV1 (+5%)</td>
<td>251</td>
<td>92</td>
<td>37</td>
<td>45</td>
<td>69</td>
<td>46</td>
<td>68</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>D ≤ 500§</td>
<td>106</td>
<td>56</td>
<td>53</td>
<td>34</td>
<td>70</td>
<td>56</td>
<td>49</td>
<td>0.21</td>
</tr>
<tr>
<td>D &gt; 500§</td>
<td>145</td>
<td>36</td>
<td>25</td>
<td>61</td>
<td>69</td>
<td>39</td>
<td>84</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Improvement (severe asthma)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FENO (-15%)</td>
<td>73</td>
<td>32</td>
<td>44</td>
<td>72</td>
<td>70</td>
<td>66</td>
<td>76</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FEV1 (+5%)</td>
<td>73</td>
<td>32</td>
<td>44</td>
<td>72</td>
<td>62</td>
<td>59</td>
<td>74</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are presented as §: ICS dose (D) in µg equ BDP.day⁻¹; &: cut-off value. N, n+ and P are the total amount of events, the amount 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. In non-severe asthma, a positive event is defined as an optimization of asthma control. A true positive event is defined as a FENO change ≤ −40% (e.g. –45%) or a FEV1 change ≥ 5% associated with an optimization of asthma control between consecutive visits. In severe asthma, a positive event is defined as an improvement of asthma control. A true positive case is defined as a FENO change ≤ −15% (e.g. –20%) or a FEV1 change ≥ 5% associated with an improvement of asthma control between consecutive visits.
Table 4: assessment of a loss of optimal asthma control or a worsening of asthma control.

<table>
<thead>
<tr>
<th>Loss of optimal control (non-severe asthma)</th>
<th>N</th>
<th>n+ (%)</th>
<th>P</th>
<th>Se (%)</th>
<th>Sp (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>FENO (+30%) &amp; Total</td>
<td>164</td>
<td>39</td>
<td>24</td>
<td>54</td>
<td>66</td>
<td>33</td>
<td>82</td>
<td>0.021</td>
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<tr>
<td>D ≤ 500§</td>
<td>104</td>
<td>19</td>
<td>18</td>
<td>74</td>
<td>64</td>
<td>31</td>
<td>92</td>
<td>0.002</td>
</tr>
<tr>
<td>D &gt; 500§</td>
<td>60</td>
<td>20</td>
<td>33</td>
<td>35</td>
<td>70</td>
<td>37</td>
<td>68</td>
<td>0.39</td>
</tr>
<tr>
<td>FEV₁ (-10%) &amp; Total</td>
<td>164</td>
<td>39</td>
<td>24</td>
<td>36</td>
<td>88</td>
<td>48</td>
<td>81</td>
<td>0.075</td>
</tr>
<tr>
<td>D ≤ 500§</td>
<td>104</td>
<td>19</td>
<td>18</td>
<td>21</td>
<td>91</td>
<td>33</td>
<td>84</td>
<td>0.51</td>
</tr>
<tr>
<td>D &gt; 500§</td>
<td>60</td>
<td>20</td>
<td>33</td>
<td>45</td>
<td>88</td>
<td>64</td>
<td>76</td>
<td>0.062</td>
</tr>
</tbody>
</table>

Worsening (severe asthma)

| FENO (+15%) &                            | 87 | 25     | 29 | 52     | 67     | 39      | 78      | 0.008|
| FEV₁ (-5%) &                             | 87 | 25     | 29 | 57     | 78     | 50      | 81      | <0.001|

Data are presented as §: ICS dose (D) in µg equ BDP.day⁻¹; &: cut-off value. N, n+ and P are the total amount of events, the amount of positive events, 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. In non-severe asthma, a positive event is defined as a loss of optimal asthma control. A true positive event is defined as a FENO change ≥ 30% or a FEV₁ change ≤ −10% (e.g. −15%) associated with a loss of optimal asthma control between consecutive visits. In severe asthma, a positive event is defined as a worsening of asthma control. A true positive case is defined as a FENO change ≥ 15% or a FEV₁ change ≤ −5% (e.g. −10%) associated with a worsening of asthma control between consecutive visits.
**Legend to the figure**

**Figure 1**: Panel A: ROC curves characterizing the ability of FENO to assess an optimization of asthma control in non-severe asthma. The solid line represents the whole population; the dashed and dotted lines represent patients treated with low and high-to-medium ICS dose, respectively. Closed circles correspond to a cut-off value of –40% change in FENO. Panel B: same as Panel A for FEV₁. Closed circles correspond to a cut-off value of 5% change in FEV₁.