Eur Respir J 2008; 32: 344–349
DOI: 10.1183/09031936.00135907

Exhaled nitric oxide predicts lung function decline in difficult-to-treat asthma

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ABSTRACT: A subset of patients with asthma is known to have progressive loss of lung function despite treatment with corticosteroids. The aim of the present study was to identify risk factors of decline in forced expiratory volume in one second (FEV_1) in patients with difficult-to-treat asthma.

In total, 136 nonsmoking patients with difficult-to-treat asthma were recruited between 1998 and 1999. Follow-up assessment was performed 5–6 yrs later in 98 patients. The predictive effect of clinical characteristics and inflammatory markers were analysed at baseline (asthma onset and duration, atopy, airway hyperresponsiveness, blood and sputum eosinophils, and the fraction of nitric oxide in exhaled air (F_{ENO})) on subsequent decline in post-bronchodilator FEV_1.

Patients with high F_{ENO} ($\geqslant$20 ppb) had an excess decline of 40.3 (95% confidence interval (CI) 7.3–73.2) mL·yr^{-1} compared to patients with low F_{ENO}. F_{ENO} $\geqslant$20 ppb was associated with a relative risk of 1.9 (95% CI, 1.1–2.6) of having an accelerated ($\geqslant$25 mL·yr^{-1}) decline in FEV_1. In patients with baseline FEV_1 $\geqslant$80% of predicted, this relationship was even stronger: 90 *versus* 29% had accelerated decline in FEV_1 (F_{ENO} $\geqslant$20 ppb *versus* F_{ENO} $<$20 ppb respectively; relative risk 3.1 (95% CI, 1.7–3.4).

Exhaled nitric oxide is a predictor of accelerated decline in lung function in patients with difficult-to-treat asthma, particularly if forced expiratory volume in one second is still normal.

KEYWORDS: Airway obstruction, asthma, nitric oxide, severity of illness index

In the majority of patients, asthma can be controlled with inhaled corticosteroids, which are the cornerstone of treatment for asthma. However, ~5–10% of all asthma patients are refractory to even high doses of inhaled or oral corticosteroid therapy [1], and may develop persistent airway obstruction over the years [2, 3], which has been associated with increased morbidity and mortality [4]. Therefore, it is of critical importance to identify patients who are less responsive to steroid treatment and are at risk of developing persistent airway obstruction at an early stage. These patients should be closely monitored and considered for novel anti-asthma drugs in order to prevent progression of their disease [5].

Persistent airway obstruction in asthma is believed to be a consequence of structural and functional changes in the airways [6], possibly related to abnormal injury and repair responses of the bronchial epithelium, which are either inherited or acquired [7]. Genetic [7] and environmental factors [2] have indeed been associated with an accelerated decline in lung function in asthma.

Recent evidence suggests that airway inflammation *per se* may be an important contributor to progressive loss of lung function. In longitudinal studies, accelerated decline in forced expiratory volume in one second (FEV_1) has been associated with severe asthma exacerbations [8] and CD8-positive T-cells in bronchial biopsies [9]. Cross-sectional studies in severe asthma have shown associations between persistent airway obstruction and eosinophilia in blood [10], sputum [3] and bronchial biopsies [11].

The aim of the present study was to assess the rate of lung function decline and identify the risk factors of accelerated decline in patients with difficult-to-treat asthma. In total, 136 nonsmoking adults with difficult-to-treat asthma were recruited between 1998 and 1999 to participate in a study aimed at identifying different clinical phenotypes of asthma and risk factors of accelerated decline in lung function [3, 12, 13]. This group of patients was reassessed 5–6 yrs later. Potential risk factors, including patients' clinical characteristics (age of asthma onset, duration of asthma, atopy and airway hyperresponsiveness), and three different noninvasive markers of airway inflammation (eosinophils in peripheral blood and induced sputum, and the fraction of nitric oxide in exhaled air (F_{ENO})) were assessed at baseline and related to the change in lung function over time.

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Received:
October 15 2007
Accepted after revision:
May 07 2008

STATEMENT OF INTEREST
Statements of interest for I.H. van Veen, P.J. Sterk, S.A. Gauw and K.F. Rabe can be found at www.erj.ersjournals.com/misc/statements.shtml

European Respiratory Journal
Print ISSN 0903-1936
Online ISSN 1399-3003

METHODS

Subjects

Between 1998 and 1999, 136 patients with difficult-to-treat asthma were recruited to participate in the study [3, 12, 13]. Pulmonologists from two teaching and eight nonteaching hospitals in the Netherlands were asked to identify nonsmoking patients with difficult-to-treat asthma from their outpatient clinic. In total, 152 patients were approached by the study coordinator by telephone and asked to participate. Of these, 16 patients refused to participate, mainly for reasons of lack of transport or time.

Patients had to fulfil the criteria for ''difficult/therapy-resistant asthma'' as defined by a European Respiratory Society Task Force [14]. All patients had a history of episodic dyspnoea and wheezing, a documented reversibility in FEV1 of >12% of the predicted value or airway hyperresponsiveness to inhaled histamine. The patients were treated with high doses of inhaled corticosteroids ($\geq$1,600 μg·day^{-1} of beclomethasone or equivalent) combined with long-acting bronchodilators for >1 yr. All patients were symptomatic and had at least one severe exacerbation during the past year requiring a course of oral corticosteroids, or were receiving chronic oral corticosteroid therapy. The maximum smoking history permitted was 10 pack-yrs.

Patients were reassessed to determine the change in lung function over time 5–6 yrs later. Patients had to be clinically stable without asthma exacerbations for $\geq$1 month before their laboratory visits. Assessment visits were postponed when patients were prescribed prednisone courses or antibiotic treatment for asthma exacerbations in the month prior to the visit.

The cross-sectional results of the present study have been previously described [3, 13]. The study was approved by the Ethics Committee of the Leiden University Medical Center (Leiden, the Netherlands) and all other participating hospitals. All patients gave written informed consent.

Design

Patients underwent an extensive assessment protocol in 1998 or 1999. Patient characteristics (age, sex, atopic status, age of asthma onset and asthma duration), lung function (pre- and post-bronchodilator FEV1, inspiratory vital capacity (IVC), airway hyperresponsiveness, lung volumes and diffusion capacity), F_{eNO} and eosinophils in peripheral blood and induced sputum were measured. A computed tomography scan of the paranasal sinuses and a 24-h pH measurement of the oesophagus were performed, and psychological questionnaires were completed. The results of these tests (with the exception of sputum eosinophils and F_{eNO}) were reported to the individual chest physician of each patient, who, if necessary, initiated treatment for previously unidentified aggravating or comorbid factors. The patients were closely monitored and treated by their own chest physician between 1998/1999 and 2004/2005. In 2004/2005, a short medical history was taken and spirometry was performed before and after maximal bronchodilation. The same lung function equipment and standardised methods as those at baseline were used.

Measurements

History taking

All patients underwent a structured case history in order to assess patient characteristics, including severity of symptoms, medication usage and duration of asthma [3]. The latter was estimated from the first-ever attack of dyspnoea or wheezing.

Atopic status and peripheral blood eosinophils

Atopic status was assessed on a score of 0–4 by specific immunoglobulin E to a panel of common aero-allergens (UniCAP; Pharmacia and Upjohn, Uppsala, Sweden). Eosinophils in blood were measured by a standard automated cell counter.

F_{eNO} in exhaled air

F_{eNO} measurements were performed according to a standardised method [15], using a chemiluminescence analyser (Sievers NOA 270B; Sievers, Boulder, CO, USA). After inhaling ''NO-free'' air (<2 ppb) from residual volume to total lung capacity, subjects performed a slow expiratory vital capacity manoeuvre with a constant expiratory flow rate of 100 mL·s^{-1} (standard at the time of study initiation). Plateau levels of F_{eNO} against time were determined and expressed as ppb.

Spirometry and histamine provocation testing

FEV1 and slow IVC measurements were performed before and 30 min after inhalation of 400 μg salbutamol and 80 μg ipratropium bromide through a volume spacer, according to standard methods. Predicted values of FEV1 and IVC were obtained from a previous study [16].

The annual decline in lung function was calculated in mL·yr^{-1} by subtracting the 2004/2005 post-bronchodilator FEV1 from the 1998/1999 post-bronchodilator FEV1. Post-bronchodilator FEV1 was chosen rather than pre-bronchodilator FEV1 to avoid the influence of variable smooth muscle contraction in the assessment of FEV1 decline.

Airway responsiveness to histamine, expressed as the provocative concentration causing a 20% fall in FEV1 (PC_{20} histamine) was measured using the standard tidal breathing method [17].

Sputum

Sputum was induced and processed according to a validated protocol [18], using the full sample method. Normal saline solutions were inhaled three times for 5 min with frequent monitoring of FEV1.

Analysis

Linear regression was used to analyse the association between potential predicting factors and decline in FEV1 (mL·yr^{-1}). Baseline FEV1 was included in the analysis as a covariate. F_{eNO}, sputum and blood eosinophils, and PC_{20} histamine were log-transformed before analysis to achieve a normal distribution of data. Results were expressed as slope of the regression line (B) with 95% confidence interval (CI), which indicates the increase in the dependent variable per one unit increase in the independent variable.

Potential predicting and modifying factors were analysed both as continuous and dichotomous independent variables, using the following contrasts: age of asthma onset $\geq$15 *versus* <15 yr

TABLE 1 Baseline characteristics of 98 study participants and 38 nonparticipants

	Participants#	Nonparticipants
Age yr	45.1±13.1	46.2±17.2
Females	68	74
Age of asthma onset yrs	14.5 (0.5–68)	18.0 (0.5–65)
Duration of asthma yrs	18.5 (2–63)	17.0 (2–73)
Inhaled steroids μg·day^{-1}	1600 (1600–4800)	1600 (1600–6400)
Chronic oral steroids	30	41
Atopy	61	51
Post-bronchodilator FEV1 % pred	78.6±24.9	76.9±22.8
PC20 histamine mg·mL^{-1}	1.1 (0.02–8)	3.6 (0.02–8)
Blood eosinophils ×10^9	0.18 (0.01–1.35)	0.22 (0.02–1.29)
Sputum eosinophils %	1.8 (0–59.4)	0.8 (0–54.5)
***F*eNO ppb**	9.6 (2–123.8)	9.1 (2–202.3)

Data are presented as mean±SD, per cent or median (range). FEV1: forced expiratory volume in one second; % pred: % predicted; PC20: provocative dose causing a 20% fall in FEV1; *F*eNO: the fraction of nitric oxide in exhaled air. #: airway hyperresponsiveness to histamine, peripheral blood eosinophils, sputum eosinophils and *F*eNO could be measured in 47, 90, 54 and 71 patients, respectively.

TABLE 2 Relative risks for an accelerated decline# in forced expiratory volume in one second (FEV1) in all patients and in patients with baseline FEV1 ⩾80% of the predicted value

	Patients	
	All	FEV1 ⩾80 %pred
Age of asthma onset ⩾15 yr	0.8 (0.4–1.3)	0.9 (0.4–1.6)
Duration of asthma ⩾18 yr	1.0 (0.6–1.5)	0.8 (0.3–1.4)
Atopy	0.9 (0.5–1.5)	0.9 (0.4–1.5)
PC20 histamine ⩽1 mg·mL^{-1}	0.6 (0.3–1.3)	0.6 (0.2–1.5)
Blood eosinophils ⩾0.45×10^9	1.3 (0.7–2.0)	2.0 (0.9–2.6)
Sputum eosinophils ⩾2%	1.6 (0.8–2.5)	2.0 (0.8–2.8)
***F*eNO ⩾20 ppb**	1.9 (1.1–2.6)	3.1 (1.7–3.4)

Data are presented as relative risk (95% confidence interval). PC20: provocative dose causing a 20% fall in FEV1; *F*eNO: the fraction of nitric oxide in exhaled air. #: ⩾25 mL·yr^{-1}.

(median for whole group); asthma duration ⩾18 *versus* <18 yr (median for whole group); atopic *versus* nonatopic; PC20 histamine ⩽1.0 *versus* >1 mg·mL^{-1} [19]; eosinophils in peripheral blood >0.45 *versus* ⩽0.45 ×10^9·L^{-1} (normal value of local laboratory); baseline FEV1 ⩾80 *versus* <80% pred (median value for the whole group); eosinophils in induced sputum ⩾2 *versus* <2% [20]; and *F*eNO ⩾20 *versus* <20 ppb. The latter values were based on receiver operating characteristic (ROC) analysis. This analysis was used to find a cut-off value for *F*eNO that would identify patients with an accelerated decline in lung function (⩾25 mL·yr^{-1}). A cut-off point for *F*eNO with a high specificity of the test was favoured. This analysis showed that an *F*eNO level of 19.1 was associated with a sensitivity of 0.48 and a specificity of 0.80, whereas an *F*eNO level of 21.9 was associated with a sensitivity of 0.44 and a specificity of 0.82 (area under the curve 0.64). Consequently, an *F*eNO value of 20 ppb was chosen. A value of 20 ppb at 100 mL·s^{-1} corresponds to ~35 ppb at 50 mL·s^{-1} [21].

Logistic regression was used to estimate odds ratios (ORs) with 95% CIs for accelerated decline in FEV1, defined as ⩾25 mL·yr^{-1}. A decline in FEV1 <25mL·yr^{-1} was considered physiological [16]. As a substantial number of patients reached the outcome of interest, ORs were inappropriate to estimate relative risks; therefore, they were re-calculated into relative risks (RRs) [22].

RESULTS

Of the 136 patients enrolled in 1998 or 1999, 98 could be reassessed. Nine patients were lost to follow-up, nine did not consent, two had missing lung function data at baseline, 12 were too disabled by their asthma or concomitant diseases to participate in the follow-up visit, and six patients had died (one due to asthma and five due to comorbidity). There were no differences in baseline characteristics between participating and nonparticipating patients (table 1).

The median (range) follow-up interval was 5.7 (4.3–6.8) yrs. The median change in post-bronchodilator FEV1 was a decline of 12.6 mL·yr^{-1}. An accelerated decline in FEV1 (⩾25 mL·yr^{-1}) was observed in 39% of the patients. The median (range) decline in FEV1 in these patients was 54.7 (27.1–173.7) mL·yr^{-1}.

The median (range) dose of inhaled corticosteroids differed slightly between baseline and follow-up (1,600 (1,600–4,800) and 1,600 (0–12,800) μg, respectively). There was no relationship between the change in corticosteroid dose and decline in FEV1, and no difference in the median dose of oral corticosteroids between baseline and follow-up.

Association between potential predicting factors and decline in FEV1

FEV1 and *F*eNO were (weakly) associated with decline in lung function (B 0.7, 95% CI 0.1–1.3) and B 29.1, 95% CI -6.5–64.7, respectively) when analyed as continuous independent variables. None of the other factors showed any association with decline in lung function. When using contrasts in variables, *F*eNO levels ⩾20 ppb were shown to be associated with an increased decline in FEV1 compared with *F*eNO levels <20 ppb, with an excess decline of 40.3 mL·yr^{-1} in patients with *F*eNO ⩾20 ppb (B 40.3, 95%CI 7.3–73.2). Further analysis showed that patients with *F*eNO values ⩾20 ppb had a 57% risk of an accelerated decline in FEV1 (⩾25 mL·yr^{-1}) compared with 30% in patients with an *F*eNO <20 ppb (RR 1.9, 95% CI 1.1–2.6; table 2).

When investigating the interaction between baseline FEV1 and *F*eNO it appeared that baseline FEV1 modified the predictive effect of *F*eNO on decline in FEV1. Therefore, the decline in FEV1 was assessed in four separate groups, based on the level of *F*eNO (<20 *versus* ⩾20 ppb) and baseline FEV1 (<80% *versus* ⩾80% pred). Patients with both an *F*eNO ⩾20 ppb and an FEV1 ⩾80% had the greatest decline in FEV1 (median decline (range) 43.5 (14.0–173.7) mL·yr^{-1}, p=0.003 (Kruskall Wallis);

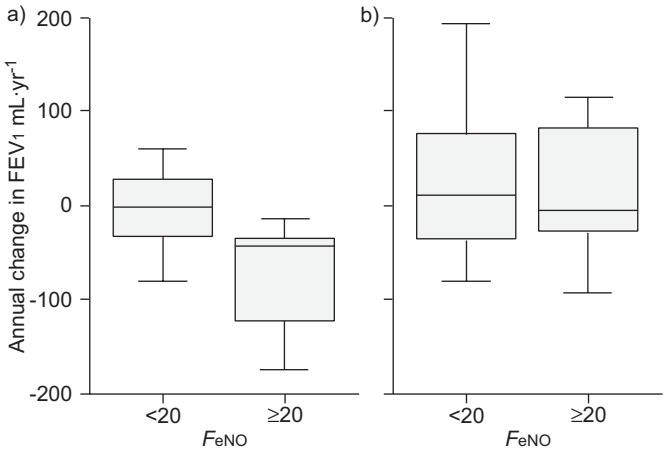

FIGURE 1. Annual change in forced expiratory volume in one second (FEV_1) after maximal bronchodilation in patients with fraction of nitric oxide in exhaled air (F_{ENO}) <20 or ⩾20 ppb and a) FEV_1 ⩾80% predicted and b) FEV_1 <80% pred. Boxes represent the median and 25th–75th percentiles, and whiskers represent the range.

fig. 1). In patients with normal baseline FEV_1 (⩾80% pred), but not in those with baseline FEV_1 <80% pred, there was a relationship between F_{ENO} and decline in FEV_1 (B 72.5, 95% CI 39.5–105.6). A 10-fold increase in F_{ENO} was associated with an additional decline in FEV_1 of 72.5 $mL \cdot yr^{-1}$ (fig. 2). Among patients with a baseline FEV_1 ⩾80% pred, those with an F_{ENO} ⩾20 ppb had a 90% risk of accelerated decline in FEV_1 compared with 29% in those with F_{ENO} <20 ppb (RR 3.1, 95% CI 1.7–3.4; table 2).

DISCUSSION

The present multicentre 5-yr follow-up study of 136 patients with difficult-to-treat asthma shows that high F_{ENO} levels predict accelerated decline in lung function. There was no association between decline in lung function and other potential predicting factors, except for baseline FEV_1. Patients with an F_{ENO} ⩾20 ppb (despite high doses of inhaled or oral corticosteroids) and FEV_1 within normal limits had a 3.1-fold risk of accelerated decline in lung function over the following 5 yrs. Elevated levels of F_{ENO} in patients with difficult-to-treat asthma might reflect an as yet undetermined injurious process in the airway wall, which is relatively unresponsive to high doses of inhaled and/or oral corticosteroids, and eventually leads to loss of lung function.

F_{ENO} is a marker of asthma that is increasingly recognised as a valuable tool in clinical practice for diagnosing and guiding treatment [23]. This noninvasive test is easy to perform, reproducible, safe and well tolerated, even in patients with severe asthma. F_{ENO} levels are increased in asthma [24], are associated with other markers of lower airway inflammation [25] and decrease in a dose-dependent manner with anti-inflammatory therapy [26]. There are, however, patients with asthma in whom F_{ENO} levels remain high, despite corticosteroid treatment [27, 28]. The present study shows that F_{ENO} >20 ppb is a predictor of a more rapid decline in FEV_1 in these patients.

In the present study, several clinical and inflammatory parameters were considered as potential predictors, but the results

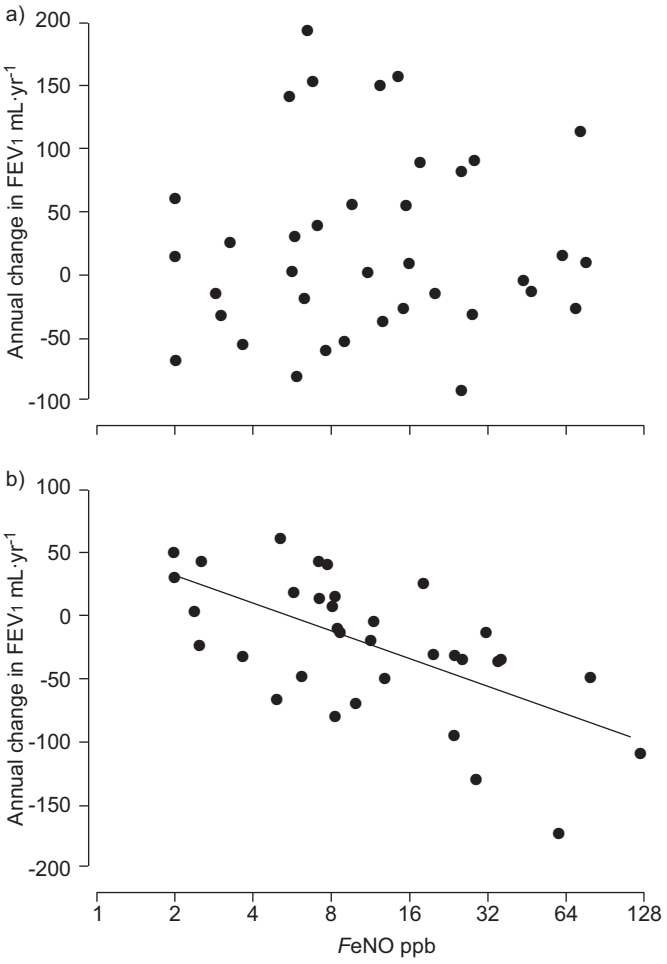

FIGURE 2. Relationship between annual change in forced expiratory volume in one second (FEV_1) after maximal bronchodilation and baseline fraction of nitric oxide in exhaled air (F_{ENO}) in a) patients with baseline FEV_1 <80% pred and b) patients with baseline FEV_1 ⩾80% pred (r= -0.62, B= -72.5; p<0.01).

showed that only elevated F_{ENO} levels were associated with accelerated lung function decline. This differs from previous studies in the overall asthma population that have shown associations between the rate of lung function decline and a short duration of asthma, nonatopic status and bronchial hyperresponsiveness [29]. The current results also differ from those of cross-sectional studies in patients with chronic severe asthma showing associations of eosinophilia in blood [10], sputum [3] and bronchial biopsy [11] with persistent airflow limitation. This can be explained by differences in the asthma populations being studied (severe as opposed to mild), or by differences in study design (longitudinal as opposed to cross-sectional). An alternative explanation for the discrepancy between the current findings and previous studies might be that some potential risk factors, such as sputum eosinophilia and airway hyperresponsiveness, could not be assessed in all the patients with difficult-to-treat asthma, which might have affected the power of the study with respect to these factors. However, the correlation coefficient between sputum eosinophils and lung function decline in the present study was only 0.09. A sample size of >1,000 patients would have been needed in order to have

obtained statistical significance with this very low correlation coefficient, which is probably not clinically relevant.

The present study may have some limitations. First, the decline in FEV1 was based on only two measurements, with an interval of 5 yrs. Although several lung function measurements have been performed in different clinics during these 5 yrs, they were not standardised with respect to equipment, premedication and asthma control, and therefore of no use for this study. For the two study visits, every effort was made to measure FEV1 with the same lung function equipment, after the same doses of inhaled salbutamol and ipratropiumbromide, and during a period of stable disease, for maximal comparability. Additionally, the choice of cut-off points for dichotomising the potential risk factors used in the analysis might be criticised. However, all cut-off points were either based on median values (age at asthma onset, asthma duration and FEV1), local normal values (blood eosinophils), recommendations from epidemiological studies (airway responsiveness and sputum eosinophils) or, in the case of *F*eNO, on ROC analysis.

How can the main findings of the present study be explained? There is increasing evidence that NO contributes to airway damage, inflammation and remodelling. In asthma, exhaled nitric oxide is mainly derived from the intrapulmonary airways. It is synthesised by constitutive and inducible NO synthases (iNOS) using L-arginine as a substrate. Airway inflammation promotes iNOS expression as well as superoxide production, interacting with NO to form the potent oxidant peroxynitrite [30]. Long-term persistence of this ''nitrosative stress'' induces cell injury and may also contribute to steroid resistance. Interestingly, inflammation-induced increase in arginase activity promotes local polyamine synthesis [30], which could induce airway remodelling, and eventually lung function decline in asthma.

Elevated levels of *F*eNO in patients with severe asthma despite corticosteroid treatment were observed. This might point towards inflammatory processes in the airways that are steroid resistant, or to insufficient doses of anti-inflammatory medication at the site of inflammation. iNOS, produced by primary human epithelial cells, is indeed not steroid sensitive [31], and severe airway inflammation may overcome the effects of steroids on iNOS expression [30]. Another possibility is that iNOS is produced in regions of the airways that are not, or barely, accessible to inhaled corticosteroids, such as the peripheral airways, or that the patients were not compliant with corticosteroid treatment. Although this latter possibility cannot be fully excluded, it is highly unlikely, as elevated *F*eNO levels have been observed in several other clinical trials where asthmatic adults receiving inhaled or oral corticosteroids were carefully evaluated [27, 28]. Taken together, iNOS expression was not sufficiently suppressed by corticosteroids, either because of (relative) corticosteroid insensitivity or inadequate steroid dosing.

The present study may have implications for clinical practice and future research. The current results suggest that *F*eNO can identify patients at risk of accelerated lung function decline at an early, ''silent'' stage of the disease. Importantly, these patients cannot be distinguished from other patients with difficult-to-treat asthma on clinical grounds or on the basis of lung function criteria. Therefore, it might be useful to include *F*eNO measurements in the assessment of patients with difficult-to-treat asthma, in order to identify those who are at risk of poor asthma outcome and those who might be eligible for novel asthma treatment or individualised treatment strategies [5, 23]. However, further confirmation of the present results is needed in a prospective follow-up study that contains a series of standardised lung function measurements over time.

In conclusion, the present authors have demonstrated that elevated levels of exhaled nitric oxide fraction predict an accelerated decline in forced expiratory volume in one second in patients with difficult-to-treat asthma, particularly if lung function is still normal. Elevated levels of exhaled nitric oxide fraction in these patients might reflect ongoing damage to the airways, and the current findings warrant further study of the mechanisms of this injurious process, which is relatively unresponsive to high doses of inhaled and/or oral corticosteroids.

ACKNOWLEDGEMENTS

The authors would like to thank M.C. Timmers (Dept of Pulmonology, Leiden University Medical Centre, Leiden, the Netherlands) for technical assistance, and the pulmonologists of the participating hospitals in the Netherlands for their cooperation: P.I. van Spiegel and G. Visschers (Slotervaart Hospital, Amsterdam); A.H.M. van der Heijden and C.H. Rikers (Rode Kruis Hospital, Beverwijk); B.J.M. Pannekoek (Reinier de Graaf Gasthuis, Delft); H.H. Berendsen, K.W. van Kralingen and J. van den Berg (Bronovo Hospital, Den Haag); H.G.M. Heijerman and A.C. Roldaan (Leyenburg Hospital, Den Haag); A.H.M. van der Heijden (Spaarne Hospital, Heemstede); H.C.J. van Klink (Diaconessenhuis, Leiden); C.R. Apap (St. Antoniushove, Leidschendam); A. Rudolphus and K.Y. Tan (St. Franciscus Gasthuis, Rotterdam).

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