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ORIGINAL ARTICLE

Clinical and inflammatory features of asthma with dissociation between fractional exhaled nitric oxide and eosinophils in induced sputum

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Abstract

Background: Measurement of the fractional exhaled nitric oxide (FeNO) and eosinophils in induced sputum are noninvasive markers for assessing airway inflammation in asthma. The clinical usefulness of the correlation between raised FeNO and sputum eosinophilia is controversial. We aimed to examine dissociation between FeNO and sputum eosinophils in a clinical series of asthma patients and to determine whether dissociation between these noninvasive markers was associated with clinical and inflammatory differences in these patients. **Methods and findings:** A total of 110 patients with asthma were included in a cross-sectional study. All of them were on maintenance treatment for asthma. All patients underwent the following on the same day: FeNO, induced sputum, spirometry, serum total IgE levels and skin prick test. The level of asthma control was determined by the Asthma control Test Questionnaire. In 46 (41.8%) patients, a discrepancy between FeNO and sputum eosinophil count was observed, of those, 34 (73.9%) had a FeNO <50 ppb and high eosinophil count, and were characterized by having a predominance of nonallergic asthma with bronchial eosinophilic inflammatory phenotype. Also, 12 (26.1%) patients had FeNO ≥50 ppb and sputum eosinophilia within the normal reference values, and were characterized by having a predominance of atopy with a paucigranulocytic inflammatory phenotype. **Conclusions:** A high percentage of patients with dissociation between results of FeNO and sputum eosinophils was observed. These patients showed differential clinical and inflammatory features.

Keywords

Asthma, asthma phenotype, eosinophils, exhaled nitric oxide, induced sputum

History

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Introduction

Assessment of airway inflammation is an important aspect in the diagnosis and clinical control of patients with asthma. Currently, asthma is considered a chronic inflammatory airway disease, and clinical practice guidelines place great emphasis on the need to monitor inflammation [1–3]. Fiberoptic bronchial biopsies have become the gold standard for measuring inflammation in the airway wall, but this is an invasive procedure not suitable for routine clinical practice, as well as for use in children and patients with severe disease. Due to the difficulties associated with this invasive method there has been a growing interest in developing less invasive methods for monitoring inflammation, notably inflammatory cells counts in induced sputum and measurement of nitric oxide (NO) in exhaled breath [4,5]. Although induced sputum has been considered the “gold standard” for phenotyping asthma [6], measurement of fractional exhaled nitric oxide (FeNO) has achieved wide acceptance in routine clinical

practice because it is easy to perform, low cost and readily available readout.

However, FeNO measurements are particularly useful to identify eosinophilic inflammation rather than neutrophilic airway inflammation, which is a prominent feature in severe asthma [7]. Furthermore, the usefulness of incorporating FeNO to usual methods to determine the level of asthma control has shown inconsistent results, with a lower reliability as compared with induced sputum [8]. Although in the clinical setting a positive correlation between FeNO and the number of sputum eosinophils may be expected in eosinophilic asthma, sometimes there is a discrepancy between the results of these methods, with high FeNO values and sputum eosinophils within reference levels and vice versa, independently of the level of asthma control and treatment. The frequency and clinical characteristics asthma patients presenting discordant FeNO and induced sputum results remains unknown. May be there are more complex mechanisms and different from the classic Th2 pathway that may explain this dissociation.

In this study, we aimed to assess dissociation between FeNO and sputum eosinophils in a clinical series of asthma patients and to determine whether dissociation between these

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noninvasive markers was associated with clinical and inflammatory differences in these patients.

Materials and methods

Study design

Between November 2011 and November 2013, a cross-sectional study was carried out in a tertiary referral university-affiliated hospital in Barcelona, Spain. The aim of the study was to determine the correlation between eosinophil count in induced sputum and FeNO in patients with asthma on maintenance treatment. Data was collected during regular clinical practice and medical procedures.

Subjects

Male and female subjects aged >18 years who fulfilled the diagnostic criteria of asthma from the American Thoracic Society [9] were recruited for the study. Asthma severity was defined according to Global Initiative for Asthma Management (GINA) [1] and the Spanish Guide for the Management of Asthma (Guía Española para el Manejo del Asma, GEMA) [2]. Patients were excluded if they had a respiratory tract infection and/or required the use of oral corticosteroids within 30 days prior to inclusion; had a low adherence to medications; had a concomitant disease potential to alter FeNO levels (sarcoidosis, lung cancer, pulmonary tuberculosis, bronchiectasis, nephropathy, rheumatic or liver disease); significant comorbidity that may affect the study results upon physician's judgment; or a cognitive impairment that could limit the comprehension or collaboration of the subject in the study.

Ethics statement

The study was conducted in accordance with the Declaration of Helsinki principles (18th World Medical Assembly) and was approved by the Clinical Research Ethics (approval number COD: 10/120/1159). All patients gave the written informed consent to participate in the study. Subjects were included upon informed consent and personal identification data were anonymized.

Assessments and study procedures

In all participants, demographic and clinical variables were recorded. On the same day the following determinations or tests were performed: FeNO, forced spirometry, inflammatory cell count in induced sputum, eosinophils count in the peripheral blood, serum total IgE levels and skin prick test to common aeroallergens in our environment. Patients also completed a Spanish validated version of the Asthma Control Test (ACT) [10].

FeNO was measured before spirometry using an electrochemical equipment (NO Vario Analyzer, FILT Lungen and Thorax Diagnostic GmbH, Berlin, Germany) and an expiratory maneuver providing a sustained 50 mL/s flow from total lung capacity, following the 2005 recommendations of the American Thoracic Society/European Respiratory Society [11]. A significant increase in FeNO was considered if the FeNO value was equal to or higher than 50 parts per billion (ppb) [8]. Spirometry was performed using a Daptospir-600

spirometer (Sibelmed, S.A., Barcelona, Spain) by an experienced pulmonary function technician and according to the 2003 recommendations of the Spanish Society of Pneumology and Thoracic Surgery (SEPAR) [12], with FEV₁ in the reference range when $\geq 80\%$ predicted [13]. Induction of sputum and sputum assay was performed as described by Pin et al. [7] and Djukanović et al. [6]. Patients with a neutrophil count $\geq 61\%$ were classified as having neutrophilic asthma, those with eosinophil sputum count $\geq 3\%$ as eosinophilic asthma, those with a neutrophil count $< 61\%$ and a eosinophil sputum count $< 3\%$ as paucigranulocytic asthma, and those with a neutrophil count $\geq 61\%$ and a eosinophil sputum count $\geq 3\%$ as mixed asthma [14]. Serum total IgE was measured by ImmunoCAP using the UniCAP 250 system (Phadia AB, Uppsala, Sweden), with increased levels for values > 160 IU/mL. Skin prick testing was performed according to a standard procedure, with positive results when the wheal diameter was > 3 mm [15]. Allergic asthma was defined as when patients had symptoms of asthma for one or more allergens and skin prick tests positive for these. Well-controlled asthma was defined as an ACT score ≥ 20 . High doses of inhaled steroids were considered as beclomethasone dipropionate (or equivalent) ≥ 1000 μ g/day [2].

Patients were classified according to the presence or absence of dissociation between FeNO and eosinophil count in induced sputum. Patients with dissociation between both methods were further subdivided into two groups: those with FeNO < 50 ppb and a high eosinophil count in induced sputum (group 1), and those with a FeNO ≥ 50 ppb and a sputum eosinophilia within the reference range (group 2).

Statistical analysis

Categorical variables were expressed as frequencies and percentages, and continuous variables as mean and standard deviation (SD). Comparisons between patients in groups 1 and 2 were performed with the Student's *t* test for quantitative variables and the chi-square (χ^2) test or the McNemar's test for categorical variables. Comparisons between the three groups of patients were performed with ANOVA. The relationship between FeNO, eosinophil count in induced sputum and eosinophil count in the peripheral blood was assessed with the Pearson's product-moment correlation coefficient. In the variables FeNO and sputum eosinophil count, log-transformation was applied. Statistical significance was set at $p < 0.05$. Statistical analysis was performed with Statistical Package for the Social Sciences version 18.0 (SPSS, Chicago, IL).

Results

Characteristics of the patients

A total of 110 patients with asthma were included in the study (50% were females), with a mean (SD) age of 49 (15) years. Never smokers accounted for 45.5% of patients, ex-smokers for 13.6% and current smokers for 9.1%. Asthma was well controlled in 92 (86.6%) patients. FEV₁ was in the reference range in 104 (94.5%) patients. Increased serum IgE levels were found in 33 (30%) patients, with a mean of 680.8 IU/mL. FeNO values were increased (FeNO ≥ 50 ppb) in 80 (72.7%)

patients. Eosinophilia in induced sputum was documented in 52 (47.3%) patients, with associated rhinitis in 68 (61.8%) patients. The skin prick test was positive in 85 (77.2%) patients. A total of 3.6% of patients were not treated with inhaled steroids. Of those treated with inhaled steroids, 42.8% received low doses of steroids, 21.4% intermediate doses and 32.2% high doses.

Dissociation between FeNO and eosinophilia in induced sputum using as FeNO values was ≥ 50 ppb

Of the 110 patients included in the study, 46 (41.8%) showed a dissociation between FeNO values and sputum eosinophilia, 34 (73.9%) of which were assigned to group 1 and the remaining 12 (26.1%) to group 2. The characteristics of patients in both groups are shown in Table 1. Patients in group 1 as compared with patients in group 2 showed a significantly higher number of visits to the emergency department in the last 12 months (mean [SD] 1.4 [2.9] versus 0, $p=0.037$), eosinophilic inflammatory (94.1%) and mixed (5.9%) phenotype versus paucigranulocytic (91.7%) and mixed (8.3%) phenotype ($p=0.001$) and lower percentages of patients with positive skin prick test (70% versus 92%, $p=0.037$).

Dissociation between FeNO and eosinophilia in induced sputum using as FeNO values was ≥ 25 ppb

Of the 110 patients included in the study, 39 (35%) showed a dissociation between FeNO values and sputum eosinophilia, 22 (56.52%) of which were assigned to group 1 and the remaining 17 (43.48%) to group 2. The characteristics of patients in both groups are shown in Table 2. When we reduce the cut-off point of FeNO to ≥ 25 ppb, no significant differences between groups were observed.

Correlations

There was a positive correlation between eosinophil count in the peripheral blood (absolute values) and eosinophil count in induced sputum ($r=0.48$, $p=0.001$). Also, there was a weak correlation between FeNO and eosinophil count in induced sputum ($r=0.288$, $p=0.016$).

The 110 patients, and using as FeNO values was ≥ 50 ppb, a 16.21% showed concordance between FeNO and induced sputum (FeNO ≥ 50 ppb/high eosinophil count in induced sputum). The characteristics of these patients are shown in online Supplementary Table 1. No significant differences were observed when this group was compared with groups 1 and 2.

Discussion

This cross-sectional study carried out in a clinical series of 110 patients with asthma on maintenance therapy showed dissociation between FeNO values and eosinophilia in induced sputum in 42% of the cases; moreover, these patients showed different clinical and inflammatory characteristics. Patients with FeNO <50 ppb and high eosinophil count in induced sputum presented predominantly eosinophilic non-allergic asthma, whereas paucigranulocytic asthma with atopy were mostly found in patients with FeNO ≥ 50 ppb and sputum eosinophilia within the normal reference values.

Different studies both in children and adults have evaluated the correlation between FeNO and eosinophilia in induced sputum, in the bronchoalveolar lavage or in the peripheral blood to assess the usefulness of FeNO for different purposes, including to evaluate asthma control, to optimize anti-inflammatory therapy or to predict response to inhaled corticosteroids [16–26]. As shown in Table 3, the results

Table 1. Demographic, clinical and functional characteristics according to the type of dissociation between FeNO and eosinophil count in induced sputum (using as FeNO values was ≥ 50 ppb).

Variables	Group 1 FeNO <50 ppb/high eosinophil count in induced sputum ($n=34$)	Group 2 FeNO ≥ 50 ppb / eosinophil count in induced sputum within reference values ($n=12$)	p Value
Females, n (%)	21 (61.8)	5 (41.7)	0.227
Age, years, mean (SD)	53.09 (16)	43.08 (13.5)	0.150
Body mass index, (kg/m ²), mean (SD)	27.04 (3.9)	24.23 (2.2)	0.107
Smoker or ex-smoker, n (%)	10 (29.4)	6 (50)	0.399
Well-controlled asthma (ATC ≥ 20), n (%)	12 (35.3)	8 (66.7)	0.455
Persistent severe asthma, n (%)	15 (44.1)	2 (16.7)	0.517
Emergency room visits in the last 12 months, mean (SD)	1.4 (2.9)	0	0.037
FEV ₁ (percent predicted), mean (SD)	80.6 (23)	88.3 (16.3)	0.422
Rhinitis, n (%)	25 (73.5)	9 (75)	0.924
Nasal polyposis, n (%)	12 (35.3)	2 (16.7)	0.350
Inhaled steroids (percent patients treated)			
High doses	31.8	33.3	0.930
Intermediate doses	22.7	16.6	
FeNO values, ppb, mean (SD)	25.35 (10.9)	85.25 (29.7)	0.001
Predominant inflammatory phenotype, n (%)			
Eosinophilic	32 (94.1)	0	0.001
Paucigranulocytic	0	11 (91.7)	
Eosinophil count induced sputum, percent, mean (SD)	11.7 (8.9)	1.03 (0.9)	0.001
Neutrophil count induced sputum, percent, mean (SD)	39.5 (20.7)	47.4 (21.4)	0.418
Skin prick test positive, n (%)	24 (70.6)	11 (91.7)	0.037
Total IgE serum, IU/mL, mean (SD)	321.4 (721.3)	572.7 (337.7)	0.734
Eosinophil count peripheral blood ($\times 10^9/L$)	0.43 (0.3)	0.32 (0.1)	0.456
Eosinophils in peripheral blood, percent, mean (SD)	5.7 (3.5)	4.5 (2.9)	0.459

Significant p value <0.05 (bold values).

Table 2. Demographic, clinical and functional characteristics according to the type of dissociation between FeNO and eosinophil count in induced sputum (using as FeNO values was ≥ 25 ppb).

Variables	Group 1 FeNO <25 ppb/high eosinophil count in induced sputum (n = 22)	Group 2 FeNO ≥ 25 ppb/eosinophil count in induced sputum within reference values (n = 17)	p Value
Females, n (%)	26 (72.7)	9 (50)	0.235
Age, years, mean (SD)	51.0 (17.1)	50.3 (15.5)	0.910
Body mass index, (kg/m ²), mean (SD)	28.5 (4.7)	25.6 (2.9)	0.080
Smoker or ex-smoker, n (%)	7 (30.7)	6 (35.7)	0.555
Well-controlled asthma (ATC ≥ 20), n (%)	12 (53.8)	13 (78.5)	0.171
Persistent severe asthma, n (%)	10 (45.5)	3 (16.7)	0.517
Emergency room visits in the last 12 months, mean (SD)	1.07 (1.8)	0.57 (1.6)	0.465
FEV ₁ (percent predicted), mean (SD)	78.2 (23.2)	82.7 (14.4)	0.551
Rhinitis, n (%)	14 (61.5)	12 (71.42)	0.445
Nasal polyposis, n (%)	5 (23)	2 (14.28)	0.462
Inhaled steroids (percent patients treated)			
High doses	23	28.57	0.749
Intermediate doses	30.7	35.7	
FeNO values, ppb, mean (SD)	16.9 (4.3)	51.92 (31.0)	0.001
Predominant inflammatory phenotype, n (%)			
Eosinophilic	22 (100)	0	0.000
Paucigranulocytic	0	12 (71.42)	
Eosinophil count induced sputum, percent, mean (SD)	12.1 (10.6)	1.04 (0.65)	0.003
Neutrophil count induced sputum, percent, mean (SD)	39.2 (17.6)	51.56 (20.3)	0.107
Skin prick test positive, n (%)	7 (30.7)	4 (21.42)	0.454
Total IgE serum, IU/mL, mean (SD)	521.0 (946.9)	338.1 (379.3)	0.510
Eosinophil count peripheral blood ($\times 10^9$ /L)	0.46 (0.3)	0.25 (0.13)	0.064
Eosinophils in peripheral blood, percent, mean (SD)	5.82 (4.1)	3.7 (2.3)	0.114

significant p value <0.05 (bold values).

Table 3. Studies providing evidence of the correlation between eosinophilia and FeNO in patients with asthma.

References	Sample type	Correlation	Observations
Strunk et al. [16]	Peripheral blood	$r = 0.51$; $p < 0.001$	144 children (aged 6–17 years) with persistent mild and moderate asthma. A correlation between FeNO, eosinophilia in peripheral blood and IgE levels was found.
Jatakanon et al. [17]	Induced sputum	$r = 0.48$; $p = 0.003$	35 patients with stable asthma. Correlation between sputum eosinophilia, FeNO and PC ₂₀ (methacholine challenge).
Mattes et al. [18]	Induced sputum	$r = 0.35$; $p = 0.09$	25 children with stable asthma and 9 healthy controls.
Jones et al. [19]	Induced sputum	$r = 0.62$; $p < 0.001$	78 patients with persistent mild and moderate asthma. Correlation between FeNO and asthma control.
Payne et al. [20]	Bronchial biopsy	$r = 0.54$; $p = 0.03$	31 children with difficult-to-control asthma. Assessment of response to oral prednisone.
Obata et al. [21]	Induced sputum	No correlation	17 adults with occupational asthma.
Piacentini et al. [22]	Induced sputum	$r = 0.438$; $p = 0.032$	25 children with persistent moderate asthma.
Warke et al. [23]	Bronchoalveolar lavage	$r = 0.78$; $p < 0.001$	71 children (29 with atopic asthma, 15 atopic healthy controls, 27 non-atopic healthy controls).
Berry et al. [24]	Bronchoalveolar lavage	$r = 0.59$; $p < 0.001$	13 healthy controls.
	Induced sputum	No correlation	25 patients with persistent mild-moderate asthma.
			27 patients with refractory asthma. Alveolar nitric oxide was measured.
Malerba et al. [25]	Induced sputum	No correlation	14 patients with persistent mild and moderate asthma treated with inhaled corticosteroids, with assessments at 3, 6 and 12 months.
	Induced sputum (at 6 months)	$r = 0.42$; $p < 0.01$	
Lim et al. [26]	Bronchial biopsy	$r = 0.14$	32 patients. No correlation between FeNO, eosinophilia in bronchial mucosa and other inflammatory and asthma control markers.

obtained are controversial from positive [16–20,22,23] to negative correlations [21,24] or even discordant results in the same study [24,25]. Our study was performed in a larger sample of asthma patients and provides evidence of a positive correlation between FeNO, sputum eosinophilia and eosinophilia in the peripheral blood. This finding indicates that results of both noninvasive methods altogether and especially FeNO have a higher clinical relevance in patients with asthma and atopy. In this respect, FeNO measurements selectively

applied can provide reliable clinical guidance, particularly when values are low. Such values are associated with high negative predictive values (>90%). Increased FeNO levels are associated with much more modest positive predictive values (75–85%) and these are less reliable to determine eosinophilic airway inflammation [27].

In clinical practice, there is tendency to generalize the results of FeNO and induced sputum. Although both noninvasive methods are useful to assess the degree of

eosinophilic airway inflammation [28–30], bronchial cell count in induced sputum provides a more accurate approximation of airway inflammation in asthmatic patients than FeNO. Also, FeNO has a limited value in the assessment of neutrophilic airway inflammation, and confounding factors may affect FeNO value [8]. Despite the fact that measurement of FeNO is included in the diagnostic algorithm for asthma by the 2009 GEMA recommendations [2], the cut-off value has been a matter of debate because normal or low FeNO values do not exclude the presence of disease. The official ATS clinical practice guideline [8] recommends that high FeNO ≥ 50 ppb in adults and ≥ 35 ppb in children can be used to indicate eosinophilic inflammation. However, FeNO values should be interpreted cautiously with reference to the clinical context (strong recommendation, low quality of evidence). Thus, this study shows that 35% of asthmatic patients had dissociation between FeNO values and sputum eosinophilia (with FeNO ≥ 25 ppb). Differences between groups were not observed. However, using the cut-off of FeNO ≥ 50 ppb, 41.8% of patients showed dissociation between FeNO values and eosinophilia in induced sputum and in this case, differences between groups reached statistical significance. So, this analysis showed that significant differences were found in the percentage of patients with nasal polyposis, dose of inhaled corticosteroids, degree of pulmonary obstruction or eosinophil count in the peripheral blood. However, some important aspects should be noted. Group 1 (FeNO < 50 ppb and high eosinophil count in induced sputum) was characterized by a lower percentage of atopy (70.3%), but with a predominant eosinophilic inflammatory phenotype in the induced sputum and poor control of the disease. These results support the discussion of Brusselle et al. [31] and Wenzel [32] who emphasized that eosinophilic asthma can occur in both allergic and non-allergic patients, but whereas the origin of eosinophilia in allergic asthma is largely understood, the triggers for eosinophilia in nonallergic eosinophilia remain to be elucidated. According to these authors, two different pathways lead to eosinophilic airway inflammation in asthma. In allergic asthma, dendritic cells present allergens to CD4⁺ T cells, inducing type 2 helper T (T_H2) lymphocytes, which produce cytokines, such as interleukin-4 (IL-4), IL-5 and IL-13, and leading to IgE switching on B cells, airway eosinophilia and mucous hypersecretion. In nonallergic eosinophilic asthma, air pollutants, microbes and glycolipids induce the release of epithelium-derived cytokines, including IL-33, IL-25 and TSLP (epithelial cytokines thymic stromal lymphopoietin), which activate type 2 innate lymphoid cells (ILC2s) in an antigen-independent manner via their respective receptors (IL-17RB, ST2 and TSLPR). Activated ILC2s would produce high amounts of IL-5 and IL-13, leading to eosinophilia, mucous hypersecretion and airway hyper-reactivity.

Patients in group 2 (FeNO ≥ 50 ppb and eosinophil count in induced sputum within reference values) was 11% of the 110 patients studied and this showed a predominance of atopy, paucigranulocytic phenotype in induced sputum, better FEV₁ and younger age. These results are consistent with several studies showing that FeNO increases mainly in states of atopy associated or not with the presence of asthma (allergic and nonallergic rhinitis, urticaria, eczema) [33,34]. In our study

we observed no significant difference between groups 1 and 2 in terms of rhinitis, but we observed significant differences in the level of atopy, this being greater in group 2, a fact that could explain the differences in these patients. Another point of interest is that the results showed differential clinical and inflammatory characteristics when we use as high FeNO ≥ 50 ppb, not with FeNO ≥ 25 ppb. It may be possible that high values of FeNO are associated with increased atopy and this factor can influence the significant differences between these groups.

Among other limitations of the study we have the fact that dissociation between FeNO values and eosinophil count in induced sputum may be partly related to false negative or false positive FeNO data. Smoking may affect the results of FeNO (decreased levels) but differences in the percentage of current smokers or ex-smokers between groups 1 and 2 were not observed, although the percentage of smokers and ex-smokers was higher among patients in group 2. In addition, the results should be interpreted with caution because they have to consider that most of the study subjects had a well-controlled asthma, likewise, other confounding variables reported in the literature that may affect FeNO measurements [35], such as the FeNO measurement technique, exhalation flow, the device used, the coexistence of respiratory infections and the body mass index were also considered.

Conclusions

This study reveals that an important percentage of patients with asthma showed dissociation between results of FeNO measurements and eosinophil count in induced sputum. These discordant groups also showed differential clinical and inflammatory characteristics. Despite the limitations of this work, further studies should enhance our ability to molecularly understand these phenotypes and lead to more targeted and personalized approaches to the management of asthma.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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References

1. GINA Report. Global Strategy for Asthma and Prevention. Available from: <http://www.ginasthma.org/>.
2. Guía Española para el Manejo del Asma (GEMA 2009). Arch Bronconeumol 2009;45(Suppl 2):1–35. Available from: <http://www.gemasma.com/>.
3. British Thoracic Society/Scottish Intercollegiate Guidelines Network. British Guideline on the Management of Asthma. A national clinical guideline. Revised 2012. Available from: <https://www.brit-thoracic.org.uk/guidelines-and-quality-standards/asthma-guideline/>.
4. Gustafsson LE, Leone AM, Persson MG, Wiklund NP, Moncada S. Endogenous nitric oxide is present in the exhaled air of rabbits, guinea pigs and humans. Biochem Biophys Res Commun 1991; 181:852–857.

5. Pizzichini E, Pizzichini MM, Efthimiadis A, Evans S, Morris MM, Squillace D, Gleich GJ, et al. Indices of airway inflammation in induced sputum: reproducibility and validity of cell and fluid-phase measurements. *Am J Respir Crit Care Med* 1996;154:308–317.
6. Djukanović R, Sterk PJ, Fahy JV, Hargreave FE. Standardised methodology of sputum induction and processing. *Eur Respir J Suppl* 2002;37:1s–2s.
7. Pin I, Gibson PG, Kolendowicz R, Girgis-Gabardo A, Denburg JA, Hargreave FE, Dolovich J, et al. Use of induced sputum cell counts to investigate airway inflammation in asthma. *Thorax* 1992;47: 25–29.
8. Dweik RA, Boogs PB, Erzurum SC, Irvin CG, Leigh MW, Lundberg JO, Olin AC, et al. An official ATS clinical practice guideline: interpretation of exhaled nitric oxide levels (FE_{NO}) for clinical applications. *Am J Respir Crit Care Med* 2011;184:602–615.
9. American Thoracic Society. Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease (COPD) and asthma. *Am Rev Respir Dis* 1987;136:225–244.
10. Vega JM, Badia X, Badiola C, López-Viña A, Olaguibel JM, Picado C, Sastre J, Dal-Ré R. Validation of the Spanish version of the asthma control test (ACT). *J Asthma* 2007;44:867–872.
11. American Thoracic Society, European Respiratory Society. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. *Am Respir Crit Care Med* 2005;171: 912–930.
12. García-Río F, Calle M, Burgos F, Casan P, del Campo F, Gáldiz JB, Giner J, et al. Normativa SEPAR. Espirometría. *Arch Bronconeumol* 2013;49:338–401.
13. Roca J, Sanchis J, Agustí-Vidal A, Segarra F, Navajas D, Rodríguez-Roisin R, Casan P, et al. Spirometric reference values from a Mediterranean population. *Bull Eur Physiopathol Respir* 1986;22:217–224.
14. Magnussen H, Holz O, Sterk PJ, Hargreave FE. Noninvasive methods to measure airway inflammation: future considerations. *Eur Respir J* 2000;16:1175–1179.
15. Bernstein IL, Li JT, Bernstein DI, Hamilton R, Spector SL, Tan R, Sicherer S, et al. Allergy diagnostic testing: an updated practice parameter. *Ann Allergy Asthma Immunol* 2008;100(Suppl 3):S1–S148.
16. Strunk RC, Szeffler SJ, Phillips BR, Zeiger RS, Chinchilli VM, Larsen G, Hodgdon K, et al. Relationship of exhaled nitric oxide to clinical and inflammatory markers of persistent asthma in children. *J Allergy Clin Immunol* 2003;112:883–892.
17. Jatakanon A, Lim S, Kharitonov SA, Chung KF, Barnes PJ. Correlation between exhaled nitric oxide, sputum eosinophils, and methacholine responsiveness in patients with mild asthma. *Thorax* 1998;53:91–95.
18. Mattes J, Storm van's Gravesande K, Reining U, Alving K, Ihorst G, Henschen M, Kuehr J. NO in exhaled air is correlated with markers of eosinophilic airway inflammation in corticosteroid-dependent childhood asthma. *Eur Respir J* 1999;13:1391–1395.
19. Jones SL, Kittelson J, Cowan JO, Flannery EM, Hancox RJ, McLachlan CR, Taylor DR. The predictive value of exhaled nitric oxide measurements in assessing changes in asthma control. *Am J Respir Crit Care Med* 2001;164:738–743.
20. Payne DN, Adcock IM, Wilson NM, Oates T, Scallan M, Bush A. Relationship between exhaled nitric oxide and mucosal eosinophilic inflammation in children with difficult asthma, after treatment with oral prednisolone. *Am J Respir Crit Care Med* 2001;164: 1376–1381.
21. Obata H, Dittrick M, Chan H, Chan-Yeung M. Sputum eosinophils and exhaled nitric oxide during late asthma reaction in patients with western red cedar asthma. *Eur Respir J* 1999;13:489–495.
22. Piacentini GL, Bodini A, Costella S, Vicentini L, Mazzi P, Sperandio S, Boner AL. Exhaled nitric oxide and sputum eosinophil markers of inflammation in asthmatic children. *Eur Respir J* 1999;13:1386–1390.
23. Warke TJ, Fitch PS, Brown V, Taylor R, Lyons JD, Ennis M, Shields MD. Exhaled nitric oxide correlates with airway eosinophils in childhood asthma. *Thorax* 2002;57:383–387.
24. Berry M, Hargadon B, Morgan A, Shelley M, Richter J, Shaw D, Green RH, et al. Alveolar nitric oxide in adults with asthma: evidence of distal lung inflammation in refractory asthma. *Eur Respir J* 2005;25:986–991.
25. Malerba M, Ragnoli B, Radaeli A, Tantucci C. Usefulness of exhaled nitric oxide and sputum eosinophils in the long-term control of eosinophilic asthma. *Chest* 2008;134:733–739.
26. Lim S, Jatakanon A, Meah S, Oates T, Chung KF, Barnes PJ. Relationship between exhaled nitric oxide and mucosal eosinophilic inflammation in mild to moderately severe asthma. *Thorax* 2000; 55:184–188.
27. Taylor DR. Advances in the clinical application of exhaled nitric oxide measurements. *J Breath Res* 2012;6:047102.
28. Berry MA, Shaw DE, Green RH, Brightling CE, Wardlaw AJ, Pavord ID. The use of exhaled nitric oxide concentration to identify eosinophilic airway inflammation: an observational study in adults with asthma. *Clin Exp Allergy* 2005;35:1175–1179.
29. Lex C, Ferreira F, Zacharasiewicz A, Nicholson AG, Haslam PL, Wilson NM, Hansel TT, et al. Airway eosinophilia in children with severe asthma: predictive values of noninvasive tests. *Am Respir Crit Care Med* 2006;174:1286–1291.
30. Schleich FN, Seidel L, Sele J, Manise M, Quaedvlieg V, Michils A, Louis R. Exhaled nitric oxide thresholds associated with a sputum eosinophil count $\geq 3\%$ in a cohort of unselected patients with asthma. *Thorax* 2010;65:1039–1044.
31. Brusselle GG, Maes T, Bracke KR. Eosinophilic airway inflammation in nonallergic asthma. *Nat Med* 2013;19:977–979.
32. Wenzel SE. Asthma phenotypes: the evolution from clinical to molecular approaches. *Nat Med* 2012;18:716–725.
33. Cordeiro D, Rudolphus A, Snoey E, Braunstahl GJ. Utility of nitric oxide for the diagnosis of asthma in an allergy clinic population. *Allergy Asthma Proc* 2011;32:119–126.
34. Gupta N, Goel N, Kumar R. Correlation of exhaled nitric oxide, nasal nitric oxide and atopic status: a cross-sectional study in bronchial asthma and allergic rhinitis. *Lung India* 2014;31: 342–347.
35. Green RH, Brightling CE, McKenna S, Hargadon B, Parker D, Bradding P, Wardlaw AJ, et al. Asthma exacerbations and sputum eosinophil counts: a randomised controlled trial. *Lancet* 2002;360: 1715–1721.

Supplementary material available online
Supplementary Table 1