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LONG-TERM ADJUSTMENT OF STABLE ASTHMA TREATMENT WITH FRACTIONAL EXHALED NITRIC OXIDE AND SPUTUM EOSINOPHILS

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Current approaches to control asthma do not involve direct assessment of airway inflammation. The aim of this study is to assess whether the therapeutic adjustments of steroid treatment according to a stepwise algorithm based on sputum Eosinophils (sEos) and fractioned exhaled Nitric Oxide (FeNO) were effective in maintaining the stability of a group of stable asthmatic patients during a twelve-month follow-up. Fourteen asthmatic patients, treated for asthma according to a previously published protocol, were enrolled in the study. The patients underwent clinical evaluation, pulmonary function tests, measuring of airway hyperresponsiveness to methacholine, and determination of FeNO and sEos at visit 1. These procedures were repeated after 6 and 12 months (Visits 2 and 3, respectively). Symptoms score gradually improved during the study (p=0.008), no changes were observed in the frequency of clinical asthma exacerbations or in airway hyperresponsiveness to methacholine. At the end of the study both sEos and FeNO were significantly improved (p=0.011 and p=0.003, respectively) and at visit 3 the median steroid dose was reduced (p=0.039) in accordance with the improving of symptoms score, FeNO and sEos values. A direct relationship was observed between the difference of FeNO values and the difference of sEos registered between visits 1 and 2 (r^2=609, p<0.001) and between visits 2 and 3 (r^2=646, p<0.001). In conclusion, long-term titration of asthma inhaled steroid treatment based on sEos and FeNO values was able to provide long-term clinical stability and improvement to the asthmatic patients studied, without significant increases in the steroid dose.

Asthma is a chronic disorder characterized by airway inflammation, inducing episodes of cough, dyspnea and wheezing. The goal of asthma management is to maintain disease control (1).

The current and most used approaches to control asthma do not involve direct assessment of airway inflammation, while to date non-invasive markers of airway inflammation are available such as measurement of the eosinophils number in induced sputum (sEos) (2) and the analysis of fractional nitric oxide (FeNO) in the exhaled breath, that is a surrogate marker of airway inflammation (3-4).

The clinical application of inflammatory biomarker analysis in titration asthma anti-inflammatory treatment to achieve clinical control is still debated, above all as to the practical feasibility of the method of the induced sputum and the individualization of clear values of cut-off for the analysis of FeNO (5-7)

A previous survey has been published in which

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the anti-inflammatory treatment of fourteen unstable asthmatic patients was titrated according to FeNO and sEos values during a twelve-month period (8); the results of the study suggested that this approach was superior in the long-term control of asthma compared to a more conventional approach based on symptoms score and lung function. We therefore decided to protract the survey for a further 12 months to verify the efficacy of this approach in patients with stable asthma. In particular, it was assessed whether the therapeutic adjustments based on the monitoring of these two biomarkers were effective in maintaining the stability of the asthmatic patients studied.

MATERIALS AND METHODS

Patients

Fourteen patients with mild-to-moderate persistent asthma [6 men, 8 women; mean age (range): 44.9 (29) years] were recruited at the Respiratory Medicine Unit of the Department of Internal Medicine (University of Brescia). The diagnosis of asthma was made according to a clinical history (dry cough, wheezing, and shortness of breath), pulmonary functional parameters, and presence of airway hyperresponsiveness to methacholine. Asthma severity was based on GINA guidelines (1). We excluded subjects with conditions that could affect FeNO or sEos measurement for reasons other than asthma, such as patients with symptoms of respiratory tract infection in the previous 6 weeks or with systemic manifestations of atopy (rash, digestive symptoms), and patients who had received treatment with oral corticosteroids during the previous 4 weeks. Other exclusion criteria were a history of cardiovascular, hepatic, renal, or neurologic diseases; lung diseases such as cystic fibrosis, bronchiectasis, chronic obstructive pulmonary disease, or respiratory failure; and therapies with drugs such as β-blockers, angiotensin-converting enzyme inhibitors, and antidepressants. Eight patients were non-smokers, and six patients had been ex-smokers for at least 2 years. Atopy was defined as the presence of skin reactivity and was assessed by skin-prick tests (nine patients were positive to the major aeroallergens in our country). All the patients enrolled agreed to participate voluntarily and gave written informed consent. The institutional review board of our hospital approved the study.

Symptom scores

Symptom scores were obtained from diary cards (9). Mean daily symptom scores (dyspnea, wheezing, cough, daytime and nighttime awakenings, each scored 0 to 3), the use of rescue short acting β2-agonists, and the percentage of symptom-free days were calculated each visit. During the visits, the clinical evaluation was referred to the period between each visit.

Study design

The study was open label without a control group. During the 1st visit the patients, treated for asthma according the protocol previously published, underwent a clinical evaluation (with specific a questionnaire including symptom score, nighttime awakenings, bronchodilator use, exacerbations, limitation to activity) and pulmonary function tests. The measure of airway hyperresponsiveness to methacholine, and determination of FeNO and sEos counts were also performed. These procedures were repeated after 6 and 12 months (visit 2 and visit 3 respectively; the flow chart of the study is shown in Fig. 1).

During visit 1, visit 2 and visit 3 the treatments for the following 6 months were prescribed according to the FeNO and sEOS values observed, using a stepwise fashion as listed in Fig. 1. As described before, symptoms and bronchodilator use, when increased, were taken into account.

Assessment of lung function

Spirometry and maximal full flow-volume curve were obtained using a pneumotachograph with volume integrator (CAD/Net system 1070; Medical Graphics Corporation, St. Paul, MN, USA). Static lung volumes were measured by means of the multibreath nitrogen washout method. The pulmonary function tests (PFTs) were performed following American Thoracic Society criteria (10). Indexes were expressed as percentage of predicted normal.

Airway Hyperresponsiveness (AHR)

The methacholine challenge was performed according to international guidelines as a dose-response curve by increasing (doubling) doses of methacholine chlorohydrate (starting with 12.5 μg) every 3 min. The test was stopped when the highest dose (1.600 μg) was tolerated, or if a fall > 20% in forced expiratory volume in the first second (FEV_1) from baseline (saline solution) was induced after methacholine inhalation. The results were expressed as the cumulative dose of methacholine provoking a 20% fall in FEV_1 (PD20). A methacholine challenge result was considered positive if the PD20 was < 1.600 μg (11).

Fractional eNO measurements

The level of FeNO was determined with a high-

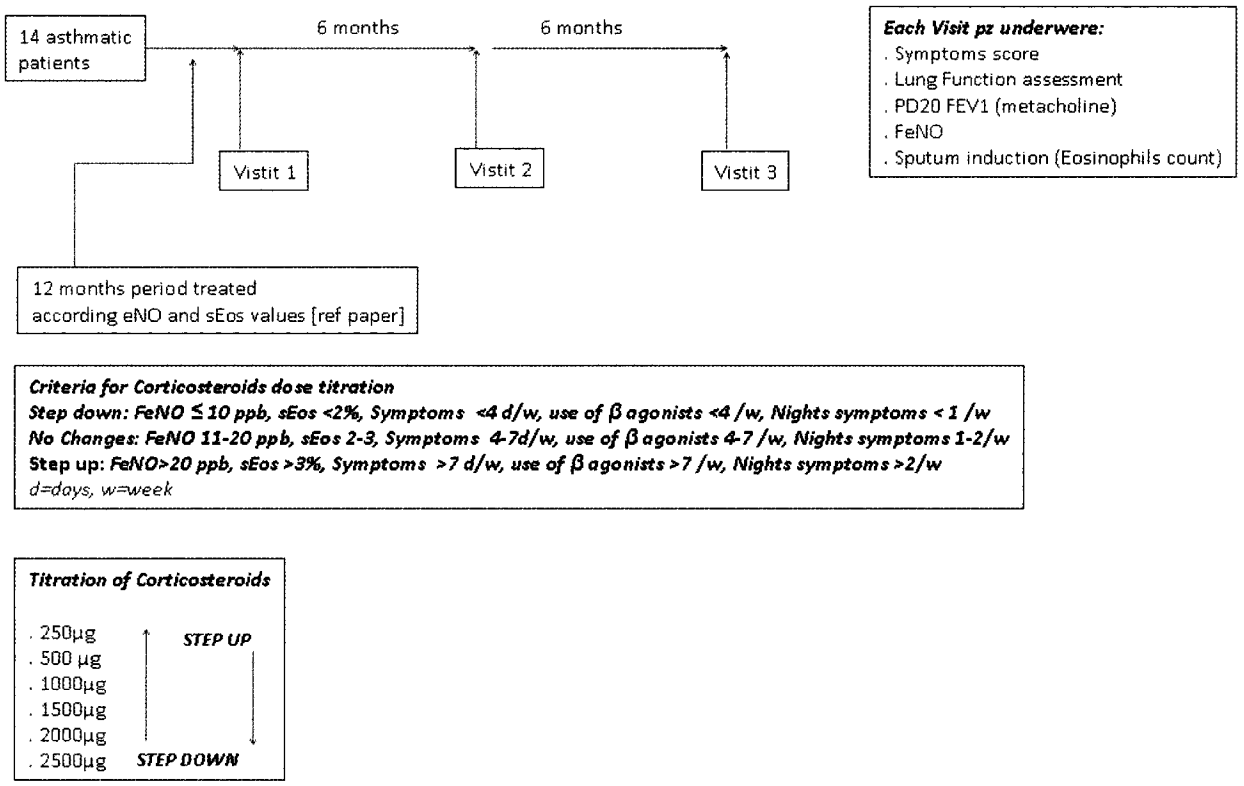

Fig. 1. *Flow chart of the study*

resolution chemiluminescence nitric oxide analyzer (Ecomedics AG CLD88; Ecomedics; Durnten, Switzerland), the limit of detection of which was 0.06 pars per billion (ppb), with measurement up to 100 ppb. Measurements were performed in accordance with American Thoracic Society recommendations using a standardized procedure for the online measurement of FeNO in adults (12), as previously described in detail (8). Values of FeNO included from 4 to 20 ppb were considered within normal limits as based on published literature identifying at 20 ppb as the cut-off point for a positive result to titration of anti-asthmatic therapy (13). For correlations, we used the variation of FeNO values as percentage change, assuming as optimal lower limit, hence 100% of potential reduction, the mean normal value of FeNO obtained in a large cohort of healthy subjects in our laboratory, amounting to 11 ppb.

Sputum induction procedure

After baseline FEV_1 and forced vital capacity (FVC) measurements, subjects were pretreated with inhaled salbutamol (200 µg by metered-dose inhaler), and 10 min later hypertonic (4.5%) sterile saline nebulized solution was inhaled for three periods for a maximum of 5 min by means of an ultrasonic nebulizer (Ultraneb 2000; DeVilbiss; Somerset, PA, USA). The subjects were instructed to cough sputum into containers. If any symptom occurred, nebulization was discontinued. The sputum was processed as previously reported (14). The cut-off for an abnormal result was defined when sEos count was > 3% as percentage cells (15). For correlations, we used the variation of sEos values as percentage change, assuming as optimal lower limit, hence 100% of potential reduction, a value of sEos equal to 0%.

Statistical analysis

Descriptive statistics were used to summarize the demographic characteristics of the patients. Continuous data were summarized by the arithmetical mean and range or by median and range. Repeated-measures analysis of variance, by Bonferroni correction for multiple comparisons test, was used to compare the different

variables at different times during the study when data were normally distributed. Variables not normally distributed were compared using Wilcoxon or Friedman test (when more than two). Pearson coefficient was used to test correlation between variables, Spearman coefficient when variables were not normally distributed; $p < 0.05$ was considered significant. Data were analyzed using statistical software (SPSS, version 15; SPSS; Chicago, IL, USA). For the main variables considered the power of the study was found to be greater than 95%.

Study outcome variables

The incidence of asthma exacerbations and the changes in clinical symptoms score were the main outcome variables of the study. Exacerbations were defined as a worsening of symptoms requiring increased use of short-acting β2-agonists by four extra puffs a day for at least 48 hours, or by nocturnal wakening, or early morning symptoms two or more times in 1 week, with or without a reduction in FEV_1 of at least 20%. Moreover, the values of PD20, FEV_1, and the variations in the inhaled corticosteroid (ICS) dose were the other outcome variables.

RESULTS

Demographic and clinical characteristics of the patients studied are listed in Table I The results are listed in Table II. During the survey period of twelve months the functional parameters of the studied patients did not change significantly: FEV_1 median (range): 99.5 (38), 98 (38), 100 (38) % of predicted at Visit 1, Visit 2 and Visit 3 respectively, $p>0.05$; FVE_1/FVC median (range): 94 (25), 92 (25), 93 (25) % at Visit 1, Visit 2 and Visit 3 respectively, $p>0.05$

A significant difference in the symptoms score was observed, it gradually improved during the study (Clinical score, median (range): 10 (9), 8.5 (11), 8 (3) at Visit 1, Visit 2 and Visit 3, respectively, $p=0.008$). Individual and mean changes of clinical score are shown in Fig. 2a.

No changes were observed in the frequency of clinical asthma exacerbations (3, 4 and 3 exacerbations at visit 1, visits 2 and 3, respectively). During the study no statistically significant changes were observed in AHR expressed as PD_{20} FEV_1 (AHR geometrical mean (range): 714.5 (1571.3), 995.8 (1439), 877 (1480) μg at visit 1, visits 2 and 3, respectively, $p>0.05$)

We observed at first an increase in inflammatory biomarkers values during the first 6 months of the study (FeNO median (range): 20.7 (40.6), 26.1 (59.8) ppb at visit 1 and visit 2, respectively $p=0.22$; sEos mean (range): 2.7 (10), 3.6 (11) % at visit 1 and visit 2, respectively $p=0.07$) that, after the proper changes of anti-inflammatory treatment, during the second six months of the study FeNO and sEos values significantly decreased (FeNO median (range): 19.8 (25.5) ppb; sEos mean (range): 1.5 (7) %, $p=0.011$ and $p=0.003$ respectively) (Fig. 2, b and c).

Beclometasone equivalent median dose at the beginning of the survey was 1000 μg (range: 2000 μg); this dose was prescribed during the previous survey according to FeNO, sEos and clinical data registered 6 months before visit 1.

At visit 1 the anti-inflammatory treatment was reduced to a median dose of 500 μg (range 2250 μg), according to the low levels of FeNO and sEos registered, then at visit 2 an unexpected elevation of FeNO and sEos values suggested an increase of steroid mean dose to a median dose of 750 μg (range: 2250 μg). Interestingly, analyzing the individual data, the worsening of inflammatory biomarkers was observed in 5 subjects while in the other 9 subjects were stable or improving; these 5 subjects were the same patients who had undergone a step-down dose of ICS during Visit 1 (data not shown). At visit 3 the median steroid dose was reduced (500 μg, range:2250 μg) due to the improvement in symptoms score and in FeNO and sEos values.

A direct relationship was observed involving the difference between FeNO values (FeNOΔ1) and the difference between sEos values (sEosΔ1) registered at visits 1 and 2 (Fig. 3).

This relationship was confirmed inking the difference between FeNO values (FeNOΔ2) and the difference between sEos values (sEosΔ2) registered at visits 2 and 3 (Fig. 3).

DISCUSSION

The results of the present study demonstrate that titrating anti-inflammatory therapy according to both FeNO and sEos levels in adult patients with stable asthma produces a fine long-term clinical control. The main result of this study is the progressive improving of clinical score observed during the twelve-month period of follow-up. Furthermore, the

Table I. *Demographic and clinical characteristics of the patients studied.*

Characteristics	Data
Patients	14
Male / Female gender	6 / 8
Mean age, yr	44.9 (29)
Height, cm	165.3 (25)
Weight, Kg	67.2 (20)
Smoking status Non-smokers Ex-smokers	 8 6
Allergy	9

Data are expressed as mean and range or N° of patients.

Table II. *Results of the tests performed during the study.*

Variables	- 6 months (16)	Visit 1	Visit 2 (6 months)	Visit 3 (12 months)	p Value
Clinical score		10 (9)	8.5 (11)	8 (3)	0.008
Exacerbations n.		3	4	3	n.s.
FEV_1, % predicted		99.5 (38)	98 (38)	100 (38)	n.s.
FEV_1/FVC%		94 (25)	92 (25)	93 (25)	n.s.
PD20 µg *		714.5 (1571.3)	995.8 (1439)	877 (1480)	n.s.
Beclometasone equiv. µg	1000 (2000)	500 (2250)	750 (2250)	500 (2250)	0.039
FeNO 50mL/s ppb		20.7 (40.6)	26.1 (59.8)	19.8 (25.5)	0.011
sEos count %[$]		2.7 (2.8)	3.6 (3.4)	1.9 (2.0)	0.003

Data expressed as Median (range)
** Geometrical mean (range) [$]Mean (range)*

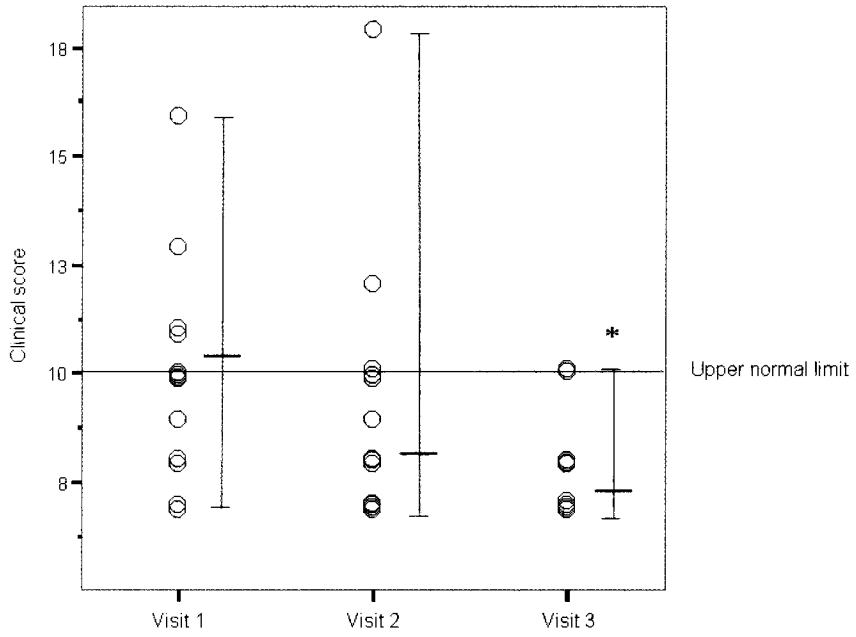

Fig. 2a. *Clinical score. Median values and range are shown. *p=0.008*

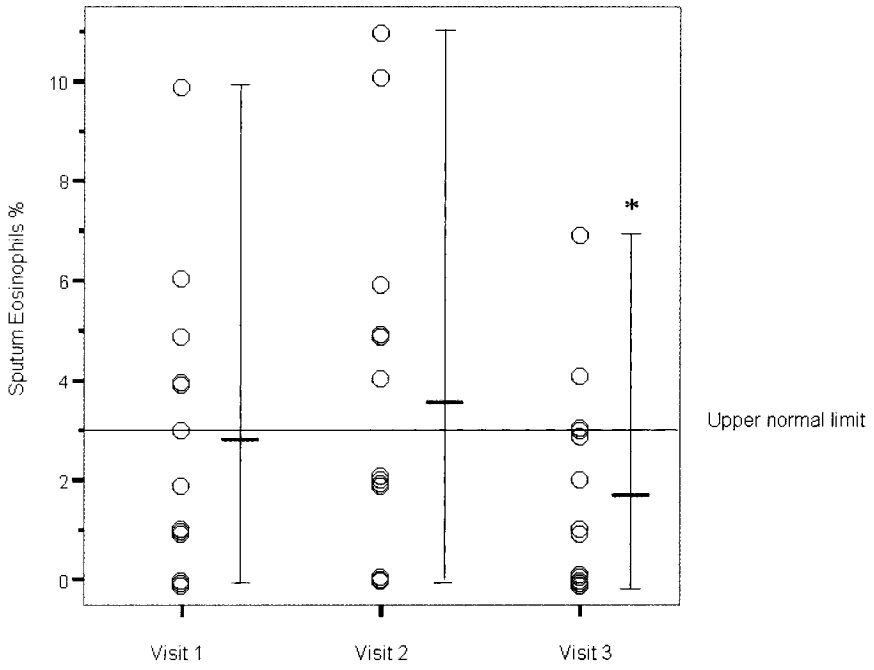

Fig. 2b. *Sputum eosinophils %. Mean values and range are shown. *p=0.011*

frequency of clinical exacerbation was stable during the whole period of the study.

Different studies have recently shown that the management of asthma treatment based on the goal to normalize the induced sputum eosinophil count results in better clinical control of asthma, observing fewer asthma exacerbations but not evident improvement of clinical symptoms control (16-17). The results of the present study confirm the conclusions of the cited papers but underline some distinctions. Firstly,

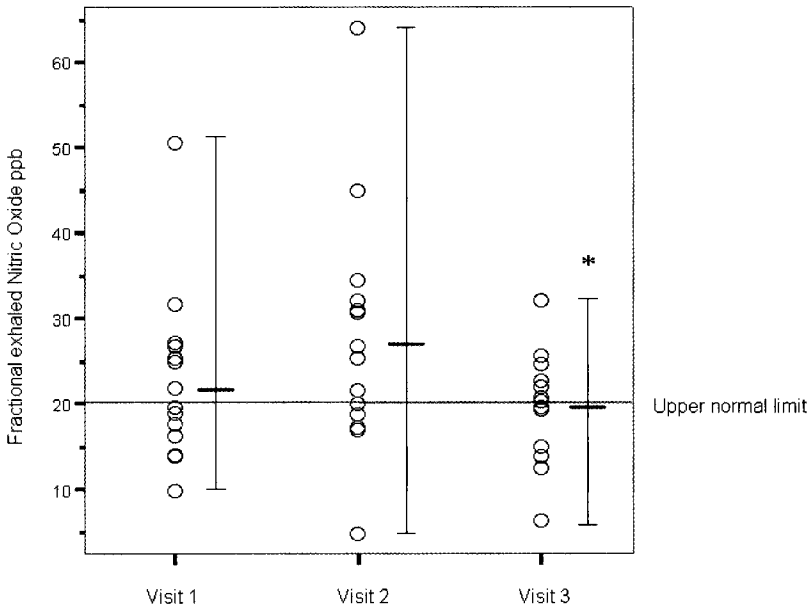

Fig. 2c. *FeNO values (ppb). Median values and range are sown. *p=0.003.*

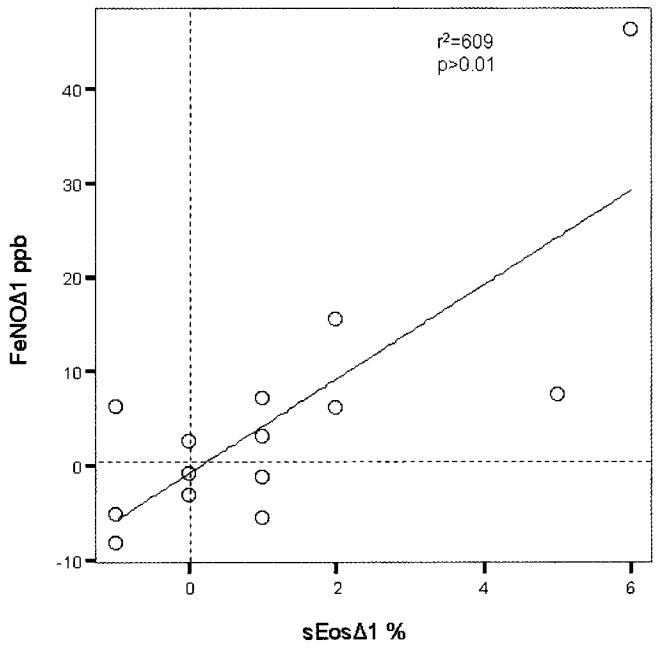

Fig. 3. *Relationship between differences of FeNO at Visit 1 and Visit 2 (FeNOΔ1) and differences of sEOS at Visit 1 and Visit 2 (sEosΔ1).*

the number of the clinical exacerbations did not change during the follow-up period of our study. It must be considered that at baseline the exacerbation rate was very low (3/14) probably because the group of patients studied had already been treated with adequate doses of corticosteroids during the previous year and were in stable clinical conditions, therefore we did not expect a further improvement of this parameter. Secondly, the results showed a slight but steady improvement in clinical score

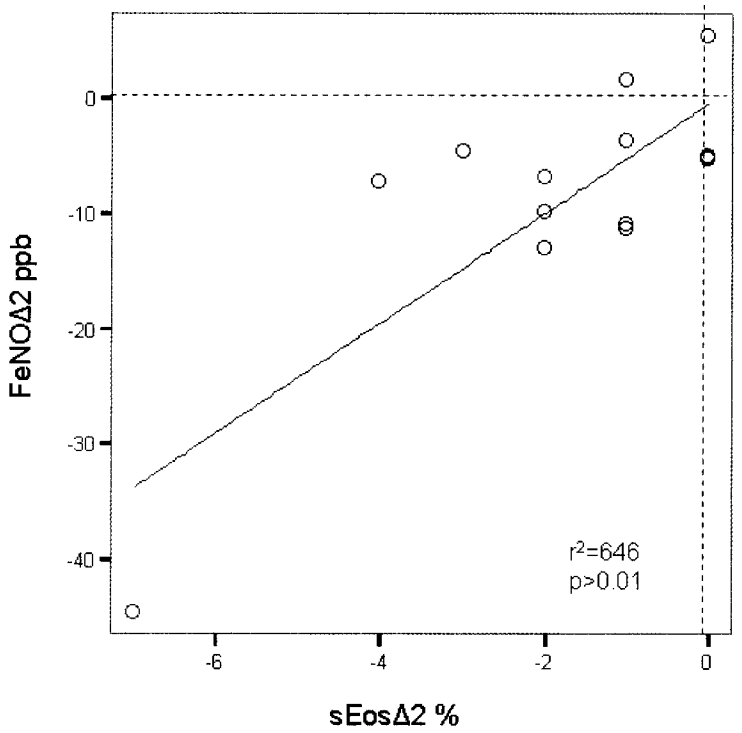

Fig. 4. *Relationship between differences of FeNO at Visit 2 and Visit 3 (FeNOΔ2) and differences of sEOS at Visit 2 and Visit 3 (sEosΔ2).*

which was not observed in other studies, probably as these showed different methodological designs and analyzed different populations of asthmatics, which are not directly comparable to the one we studied .

More uncertain in literature is the use of FeNO values to titrate asthma antiinflammatory treatment (18-23). However, a survey was recently published where asthma management in pregnancy had been improved by using an FeNO based algorithm (24). The reasons why some of the other studies cited did not find a role for FeNO in titrating asthma therapy are various, such as that some papers were focused on patients who probably did not benefit from FeNO provided information (as patients with prevalent neutrophilic airway inflammation) (25), other studies used sub-optimal cut points for up and down titration corticosteroid treatment (26, 27) and others involved particular populations such as young-adults or children. However, in one study a reduction of severe exacerbation was achieved despite an ICS mean dose reduction (18). It should be emphasized that it is precisely the combined use of the two biomarkers that seems to favor the efficacy of this approach in titrating asthma therapy. In particular, probably because the recognition of a prevalent eosinophilic airway inflammation increases the benefits supplied by the measurement of FeNO. Interestingly in our results an initial increase of FeNO and sEos values was observed between visit 1 and visit 2 after a mean step-down dose of ICS, this data did not translate to a clinical worsening of asthma symptoms, suggesting that airway inflammation had initiated before the clinical manifestations in the asthmatic patients studied; according to this point of view, it is possible to argue that the monitoring of inflammatory biomarkers is able to detect "sub-clinical" changes in airway inflammation and guide treatment before the occurring of clinical symptoms. Our data show a very close association linking the difference between the FeNO and sEos values registered during visit 1

and during visit 2 (FeNOΔ1 and sEosΔ 1) and during visits 2 and 3 (FeNOΔ2 and sEosΔ2) these results may suggest that sEos and FeNO changes are correlated, and that the individual differences of FeNO and sEos may have greater significance in predicting the course of inflammation of the airways rather than the fixed cut-off values, particularly during situations of clinical uncertainty: when the values of FeNO are between 20 ppb and 30 ppb and those of eosinophils between 2% and 3%. This observation could suggest a more simple and effective use of FeNO in clinical practice, as highlighted by the recent ATS clinical practice guidelines (26). In fact, assessment of FeNO values as a surrogate marker of eosinophilic airway inflammation is largely feasible in primary care and quite inexpensive. We did not observe a significant difference of bronchial hyperresponsiveness during the study, probably because most of the patients studied were under ICS treatment and showed a PD20 >1600 μg at baseline. The limitations of the study include the small number of participants and the lack of a control group, but the results in our opinion deserve to be published as they suggest a possible use of inflammatory biomarkers ,in particular of FeNO in stable asthma clinical management.

It would be worth testing this approach using other non-invasive techniques for assessing airway inflammation including measurement of markers of oxidative stress, such as 8-isoprostane (28), in exhaled breath condensate (EBC) (29), and e-nose (30). Further studies are required to clarify whether a strategy for asthma control based on inflammatory parameters could also be applied to therapeutic regimens with leukotriene receptor antagonists (31) should also be investigated.

In conclusion, the results of the study suggest that long-term titration of asthma inhaled steroid treatment according to a stepwise algorithm based on sEos and FeNO values is able to provide a long-term clinical control and improvement to the asthmatic patients with prevalent eosinophilic airway inflammation without significant increases in the dose of ICS

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