

# Exhaled Nitric Oxide in Childhood Allergic Asthma Management A Randomised Controlled Trial

Eva J. Peirsman, MD,<sup>1\*</sup> Thierry J. Carvelli, MD,<sup>2</sup> Pierre Y. Hage, MD,<sup>3</sup> Laurence S. Hanssens, MD,<sup>3</sup> Luc Pattyn, MD,<sup>4</sup> Marc M. Raes, MD,<sup>5</sup> Kate A. Sauer, MD,<sup>6</sup> Françoise Vermeulen, MD,<sup>7</sup> and Kristine N. Desager, MD, PhD<sup>1,8</sup>

**Summary.** Objective: We investigated the potential yield of incorporating fractional exhaled nitric oxide (FeNO) measurements in childhood allergic asthma management. Methods: Ninety-nine children with persistent allergic asthma were included in this multicentre, single-blind, randomized controlled trial. Treatment was based on the Global Initiative for Asthma (GINA) guidelines. In the FeNO group, asthma management was also guided by FeNO measurements. Health outcomes were evaluated over a 52-week timeframe. Results: Fewer asthma exacerbations were registered in the FeNO group. 24% of the children in the FeNO group experienced one or more exacerbations per year, compared with 48% in the clinical group ( $P=0.017$ ). The proportion of symptom-free days did not differ between groups. In the FeNO group, more months of leukotriene receptor antagonist use (median (interquartile range)) were observed: 12 (9–12) months, compared with 9 (3–12) months in the clinical group ( $P=0.019$ ). Next, the evolution of inhaled corticosteroid doses between visits 1 and 5 (median change (interquartile range)) showed a significant increase of  $+100\text{ }\mu\text{g}$  (0,  $+400$ ) in the FeNO group and a change of  $0\text{ }\mu\text{g}$  ( $-200$ ,  $+80$ ) in the clinical group ( $P=0.016$ ). Conclusions: FeNO measurements in childhood asthma management did not improve the proportion of symptom-free days, but did result in fewer asthma exacerbations associated with an increased leukotriene receptor antagonist use and an augmentation of the inhaled corticosteroid doses. **Pediatr Pulmonol.** © 2013 Wiley Periodicals, Inc.

**Key words:** airway inflammation; asthma exacerbations; inhaled corticosteroids; leukotriene receptor antagonist.

**Funding source:** Investigator Initiated Studies Program of Merck & Co., Inc.

<sup>1</sup>Laboratory of Experimental Medicine and Paediatrics, University of Antwerp, Antwerp, Belgium.

<sup>2</sup>Department of Paediatrics, Centre Hospitalier Peltzer - La Tourelle, Verviers, Belgium.

<sup>3</sup>Department of Paediatrics, Universitair Kinderziekenhuis Koningin Fabiola - Hôpital Universitaire Des enfants Reine Fabiola, Brussels, Belgium.

<sup>4</sup>Department of Paediatrics, Algemeen ziekenhuis Turnhout, Campus Sint-Jozef, Turnhout, Belgium.

<sup>5</sup>Department of Paediatrics, Jessa ziekenhuis, Hasselt, Belgium.

<sup>6</sup>Department of Paediatrics, Algemeen ziekenhuis Sint-Jan, Bruges, Belgium.

<sup>7</sup>Department of Paediatrics, Hôpital Erasme, Université Libre de Bruxelles, Brussels, Belgium.

<sup>8</sup>Department of Paediatrics, Antwerp University Hospital, Antwerp, Belgium.

K.D. was responsible for the study design and the algorithm development. E.P. analysed and interpreted data and wrote this manuscript. T.C., P.H., L.H., L.P., M.R., K.S., F.V., and K.D. contributed to the trial execution as investigators. All authors revised and approved the final version of this manuscript.

**Conflict of interest:** Research was supported in part by a research grant from the Investigator Initiated Studies Program of Merck & Co., Inc. NO analysers used in this study were provided by Aerocrine, Solna, Sweden. The study sponsors were not involved in the study design; in the data collection, analysis and interpretation; in the writing of the report; or in the decision to submit the paper for publication.

\*Correspondence to: Eva Peirsman, MD, Universiteitsplein 1, Building T Room 436, 2610 Wilrijk, Belgium. E-mail: eva.peirsman@uantwerpen.be

Received 30 November 2012; Revised 18 April 2013; Accepted 10 July 2013.

DOI 10.1002/ppul.22873  
Published online in Wiley Online Library  
(wileyonlinelibrary.com).

## INTRODUCTION

As a chronic inflammatory disease, childhood asthma is characterized by periodic attacks of wheezing, shortness of breath and coughing. According to the Global Initiative for Asthma (GINA) guidelines, asthma management should be based on the symptoms reported by the child and parents, the need for rescue medication and the pulmonary function tests.<sup>1</sup> These pulmonary function tests, such as measurements of forced expiratory volume in 1 sec (FEV<sub>1</sub>), provide indirect parameters of the airway calibre and airflow obstruction, but show little or no correlation with the underlying airway inflammation.<sup>2</sup> Additionally, the reporting of symptoms is implemented as a core element in the asthma control assessment.<sup>1</sup> However, self-reported symptoms are not always reliable, especially in children, owing to subjective perception and experience.<sup>3</sup> Moreover, these symptoms are only episodically present.<sup>1</sup> Consequently, a clear relationship does not exist between reported symptoms and the presence and intensity of airway inflammation.<sup>4,5</sup>

Airway inflammation has been recognized as an important physiopathological characteristic underlying asthma.<sup>6</sup> Since symptoms and lung function measurements poorly reflect airway inflammation, a biomarker providing direct information related to the inflammatory processes could be of great interest for asthma management.<sup>7</sup> Therefore, the analysis of induced sputum eosinophils has been proposed to assess airway inflammation. In adults, treatment adjustments based on sputum eosinophils resulted in a reduction of the exacerbation rate.<sup>8</sup> However, this technique requires expertise and is less suitable for children, due to possible discomfort.

A non-invasive approach consists of measurement of fractional exhaled nitric oxide (FeNO).<sup>9</sup> The production of FeNO by the inducible NO synthase in the lower airways is upregulated in the presence of a type 2 T-helper cell inflammation and correlates with the sputum eosinophilic count.<sup>10,11</sup> Previously, it has been shown that FeNO measurement could be helpful in monitoring of anti-inflammatory treatment, where it appears to act as a response marker to adjusted anti-inflammatory therapy.<sup>12</sup> Moreover, an increased FeNO level could indicate an uncontrolled eosinophilic inflammation and could be associated with a heightened future exacerbation risk.<sup>13</sup> Furthermore, FeNO measurements are acceptable for children and hand-held devices are widely available.<sup>14</sup>

We hypothesized that tailoring maintenance treatment with complementary FeNO measurements would improve childhood allergic asthma management thanks to more adequate therapeutic interventions and would result in a greater number of symptom-free days and fewer asthma exacerbations.

## MATERIALS AND METHODS

### Study Design

During this 52-week single-blind parallel-group effectiveness study, five visits, one visit every 3 months, were organized by physicians from seven Belgian hospitals. Inclusion criteria covered mild to severe persistent asthma according to GINA guidelines, for a period of at least 6 months, and allergic sensitization (i.e., a positive skin prick test and/or specific IgE antibodies against inhalant allergens), as it was shown that elevated FeNO values are found in the presence of allergic airway inflammation.<sup>10</sup> Exclusion criteria comprised significant comorbidity, an acute exacerbation or the administration of experimental medication 4 weeks prior to the screening visit, hospitalization and/or systemic corticosteroids 12 weeks prior to the screening visit or oral corticosteroid dependence. The study was approved by the Ethical Committees of the Antwerp University Hospital and all participating centres. Written informed consent was obtained from both parents. This trial is registered on clinicaltrials.gov as number NCT00562991.

### Algorithms

In the first algorithm, or the *clinical group*, asthma control and treatment adjustments during each visit were determined by the reporting of symptoms (i.e., limitation of activities, daytime and nocturnal symptoms), the need for rescue treatment during the two preceding weeks and spirometry (FEV<sub>1</sub>), based on the GINA guidelines.<sup>1</sup> In the second algorithm, or the *FeNO group*, FeNO measurements were primarily used to adjust the treatment (Table 1). Our goal was to keep FeNO below 20 parts per billion (ppb), the rounded 95% upper limit of FeNO values in healthy children, which was deduced from previous trials.<sup>15,16</sup> An independent research nurse, blinded for all patient characteristics, consecutively allocated the participants to the two groups, taking into account the initial FeNO measurements with the cut-off point of 20 ppb as a stratifying factor. This approach guaranteed an equal number of participants with a FeNO value below and above 20 ppb in each group. Patients were blinded in both groups and physicians were blinded to the FeNO values in the clinical group. Blinded lung function technicians performed FeNO measurements in both groups at a constant flow of 50 ml/sec using the single-breath online technique with the FeNO analyser NIOX MINO; Aerocrine, Solna, Sweden.<sup>17</sup>

### Health Outcomes

Symptom-free days were observed as the primary outcome and assessed using the first four questions from the childhood Asthma Control Test, which were

TABLE 1—Algorithms

| Clinical group                           | Controlled asthma                                                                                     | Partly controlled                                                                                         | Uncontrolled                                                                                      |
|------------------------------------------|-------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------|
| FeNO group                               | <b>NO <math>\leq</math> 20 ppb and controlled</b>                                                     | <b>NO <math>\leq</math> 20 ppb and partly controlled or uncontrolled</b>                                  | <b>NO <math>&gt;</math> 20 ppb regardless of symptoms</b>                                         |
| <i>Current treatment</i>                 | <i>Adapt</i>                                                                                          | <i>Adapt</i>                                                                                              | <i>Adapt</i>                                                                                      |
| ICS (dosage in budesonide or equivalent) | ICS step down: $-100\text{ }\mu\text{g/day}$ , below $100\text{ }\mu\text{g/day}$ : stop and add LTRA | Consider + LTRA                                                                                           | +LTRA                                                                                             |
| LTRA                                     | =                                                                                                     | Consider + ICS $100\text{ }\mu\text{g/day}$ (maximum $200\text{ }\mu\text{g/day}$ )                       | +ICS $100\text{ }\mu\text{g/day}$ (maximum $200\text{ }\mu\text{g/day}$ )                         |
| ICS + LTRA                               | ICS step down: $-100\text{ }\mu\text{g/day}$ , below $100\text{ }\mu\text{g/day}$ : stop ICS          | Consider ICS step up $+100\text{ }\mu\text{g/day}$ (maximum $400\text{ }\mu\text{g/day}$ , then add LABA) | ICS step up: $100\text{ }\mu\text{g/day}$ , (maximum $400\text{ }\mu\text{g/day}$ , then add LABA |
| ICS + LABA                               | Stop LABA                                                                                             | Consider + LTRA                                                                                           | Replace LABA with LTRA                                                                            |

Asthma treatment was adjusted every 3 months according to these algorithms. Maintenance treatment contained the use of inhaled corticosteroids (ICS), leukotriene receptor antagonists (LTRA, montelukast) and long-acting  $\beta$ -2 agonists (LABA).

completed each day.<sup>18</sup> A symptom-free day was considered to be indicated by a score of two or three on the first question and a score of three on the other questions. The occurrence of exacerbations, unscheduled asthma-related contacts, hospital or emergency room admissions, non-attendance at school and the need for a caregiver to take time off to look after his/her child were evaluated as secondary health outcomes at each visit. An exacerbation was defined as an episode of progressive increased shortness of breath, coughing, wheezing, or chest tightness, or a combination of these symptoms, as stated in GINA guidelines.<sup>1</sup>

## Statistics

Based on data from a previous study, we presumed the proportion of symptom-free days in the clinical group would be 50%.<sup>19</sup> A power calculation showed that 35 patients per group were needed to detect an increase of 45% in the proportion of symptom-free days, resulting in a proportion of symptom-free days of 72.5%, in the FeNO group, with 80% probability and a two-sided significance level of 5%. To allow for dropouts, we recruited 101 children. The Chi-square-test, the Spearman correlation, the Mann–Whitney test, the Wilcoxon Signed Ranks test and the Student's *t*-test were used, as appropriate. Statistical tests were conducted two-sided with a significance level of 5%. Multiple logistic regression models were designed to investigate the effect of FeNO measurements on the occurrence of one or more exacerbations over 52 weeks and to adjust for confounders. The participating centres, body mass index, baseline asthma control and asthma severity, exposure to cigarette smoke, presence of concurrent rhinitis and use of asthma medication were tested as confounding factors. Statistical analysis was performed with PASW Statistics for Windows, Version 18.0. SPSS Inc., Chicago, United States of America.

## RESULTS

### Study Population and Baseline Characteristics

This study was carried out from October 2007 to November 2009 with enrolment of allergic asthmatic children from October 2007 to November 2008 (Fig. 1). A total of 101 children aged 5–14 years were invited. Six children, or 6.1%, dropped out, all due to loss of interest. Baseline characteristics are described in detail in Table 2 and showed no significant differences between the two groups.

### Primary Outcome

We found no significant difference between the groups regarding the proportions of symptom-free days (Table 3).

Due to a considerable amount of missing data regarding this primary outcome, we chose to define a completion rate of  $>85\%$  (or data from 310 out of 365 days) as reliable. For 83 children, data was successfully recorded from  $>310$  days. This means that 10 children, five from each group, provided us with incomplete data due to

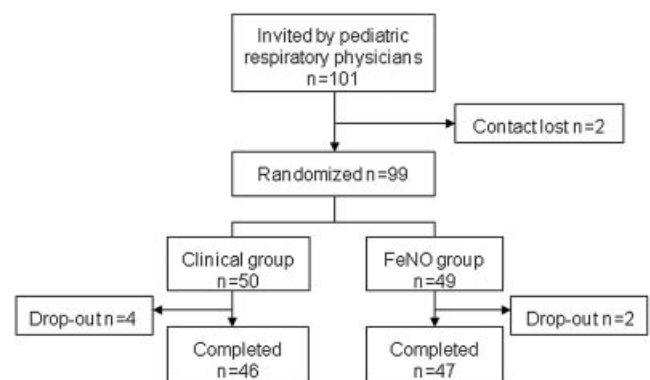


Fig. 1. Participant flow. Contact was lost in two children. Fifty children were assigned to the clinical group and 49 children to the FeNO group.

TABLE 2—Baseline Characteristics

|                                                                                 | Clinical group (n = 50)       | FeNO group (n = 49)                                    | P-value |
|---------------------------------------------------------------------------------|-------------------------------|--------------------------------------------------------|---------|
| Gender, n male/female <sup>1</sup>                                              | 33/17                         | 33/16                                                  | 0.89    |
| Age, year <sup>3</sup>                                                          | 10.7<br>(2.1; 6.3–14.3)       | 10.6 (2.2; 5.2–14.2)                                   | 0.91    |
| Weight, kg <sup>3</sup>                                                         | 36.7<br>(10.7; 20.0–72.3)     | 36.2 (10.2; 18.0–69.0)                                 | 0.80    |
| Height, cm <sup>3</sup>                                                         | 143.2 (13.9; 117.0–179.2)     | 141.4 (14.4; 109.0–167.0)                              | 0.52    |
| Asthma severity: <sup>1</sup> mild, moderate, severe, n (%)                     | 14 (28.0), 33 (66.0), 3 (6.0) | (1 missing value),<br>14 (28.6),<br>32 (65.3), 2 (4.1) | 0.92    |
| Asthma control: <sup>1</sup> controlled, partly controlled, uncontrolled, n (%) | 22 (44), 22 (44), 6 (12)      | 21 (42.9), 18 (36.7), 10 (20.4)                        | 0.49    |
| Exposure to cigarette smoke, <sup>1</sup> no/yes n                              | 41/8 (1 missing value)        | 43/5 (1 missing value)                                 | 0.39    |
| Concurrent rhinitis, no/yes n <sup>1</sup>                                      | 19/30 (1 missing value)       | 12/35 (2 missing values)                               | 0.17    |
| FEV <sub>1</sub> , % predicted <sup>3</sup>                                     | 89.0 (16.2; 53.0–123.3)       | 92.9 (12.2; 67.4–125.5)                                | 0.18    |
| FEV <sub>1</sub> post bronchodilator, <sup>3</sup> % predicted                  | 96.7 (13.9; 67–127.8)         | 101.3 (11.8; 78.6–144.5)                               | 0.08    |
| FeNO, ppb <sup>2</sup>                                                          | 27.5 (16.3–47.3)              | 32.5 (13.8–72.0)                                       | 0.71    |
| Daily ICS dose, µg <sup>2</sup>                                                 | 320 (145–400)                 | 320 (160–400)                                          | 0.94    |
| LTRA use, n no/yes <sup>1</sup>                                                 | 29/21                         | 24/25                                                  | 0.37    |
| LABA use, n no/yes <sup>1</sup>                                                 | 27/23                         | 24/25                                                  | 0.62    |
| Number of children who had ≥1 exacerbation*, count/total (%) <sup>1</sup>       | 12/46 (26.1)                  | 12/48 (25.0)                                           | 0.90    |
| Number of asthma exacerbations*, count <sup>2</sup>                             | 17                            | 15                                                     | 0.86    |

Mean data (SD, range: minimum-maximum) or median (interquartile range) are displayed.

<sup>1</sup>Chi-square test.

<sup>2</sup>Mann–Whitney test.

<sup>3</sup>Student's *t*-test.

\*During the three preceding months before visit 1.

negligence, which resulted in 23% to 79% missing data in the datasets of these individual children.

## Secondary Outcomes

Significantly fewer exacerbations and unscheduled contacts were observed in the FeNO group. These two parameters were correlated (Spearman's correlation coefficient: 0.725,  $P < 0.001$ ), which could indicate that most unscheduled contacts were due to exacerbations. Moreover, fewer participants from the FeNO group experienced one or more exacerbations and required unscheduled contacts (Table 3). Comparable numbers of

children in the FeNO and clinical groups had been admitted to the hospital or emergency room, or missed school. A similar number of caregivers in both groups had to take time off during the 52-week follow-up. Four oral corticosteroid courses were prescribed in three children in the clinical group, whereas three courses were prescribed in two children in the FeNO group. Multiple logistic regression models were used to analyse the relationship between the incorporation of FeNO measurements and the occurrence of one or more exacerbations over 52 weeks (see Table 4 for best fitted model). Not implementing FeNO measurements was associated with a significantly increased risk of experiencing

TABLE 3—Main Outcome Parameters Over 52 Weeks

|                                                                                         | Clinical group   | FeNO group       | P-value |
|-----------------------------------------------------------------------------------------|------------------|------------------|---------|
| Percentage of Symptom-free days, median (interquartile range) <sup>2</sup>              | 79.6 (51.7–94.0) | 83.7 (27.1–91.9) | 0.58    |
| Number of asthma exacerbations over 1 year, count <sup>2</sup>                          | 35               | 18               | 0.02    |
| Number of unscheduled asthma-related contacts over 1 year, count <sup>1</sup>           | 28               | 14               | 0.03    |
| Number of children who had ≥ 1 exacerbation, count/total (%) <sup>1</sup>               | 22/46 (47.8%)    | 11/46 (23.9%)    | 0.02    |
| Number of children with ≥ 1 unscheduled contact, count/total (%) <sup>1</sup>           | 15/43 (34.9%)    | 6/44 (13.6%)     | 0.02    |
| Number of children with ≥ 1 hospital admission, count/total (%) <sup>1</sup>            | 1/43 (2.3%)      | 1/43 (2.3%)      | 1.00    |
| Number of children with ≥ 1 emergency room admission, count/total (%) <sup>1</sup>      | 4/46 (8.7%)      | 2/45 (4.4%)      | 0.41    |
| Number of children who missed school, count/total (%) <sup>1</sup>                      | 12/46 (26.1%)    | 10/46 (21.7%)    | 0.63    |
| Number of children whose caregiver had to take time off, count/total (%) <sup>1,*</sup> | 8/46 (17.4%)     | 6/45 (13.3%)     | 0.59    |

The chi-square test<sup>1</sup> and Mann–Whitney test<sup>2</sup> were used for the statistical analysis.

\*None of the caregivers had to take time off from work twice to look after their child.

**TABLE 4—Multiple Logistic Regression Model for the Risk of Experiencing  $\geq 1$  Exacerbation**

| Variables                                              | Unadjusted Odds ratio<br>(95% CI) | P-value | Adjusted Odds ratio<br>(95% C.I.) | P-value |
|--------------------------------------------------------|-----------------------------------|---------|-----------------------------------|---------|
| Not implementing FeNO measurements<br>(~randomization) | 2.92 (1.20–7.11)                  | .019    | 6.19 (2.03–18.85)                 | 0.001   |
| Concurrent rhinitis                                    | 2.59 (0.96–6.97)                  | .060    | 3.96 (1.27–12.31)                 | 0.017   |
| Cumulative ICS dose                                    | 1.00 (1.00–1.00)                  | .064    | 1.00 (1.00–1.00)                  | 0.066   |
| Months of LTRA use                                     | 1.09 (0.98–1.21)                  | .119    | 1.15 (1.01–1.30)                  | 0.036   |

The best fitted model is provided above, only the variables randomization, concurrent rhinitis, cumulative ICS dose and months of LTRA use were included.

exacerbations. In the same way, the presence of concurrent rhinitis generated an increased asthma exacerbation risk, as demonstrated using the multiple logistic regression model. Additionally, an association between higher LTRA use and the occurrence of exacerbations over 52 weeks was observed.

### FEV<sub>1</sub>, Reversibility and FeNO Measurements

The lung function was examined at each visit. At visits 1 and 5, reversibility in FEV<sub>1</sub> after administration of 200  $\mu$ g short acting  $\beta$ -agonists was included. No differences were

revealed between the two groups. Moreover, in both groups FEV<sub>1</sub> and reversibility in FEV<sub>1</sub> remained stable between visits 1 and 5 (E-Tables 1–3). In the clinical group, FeNO values exhibited no significant changes over the five visits. In the FeNO group, an evolution toward lower FeNO values was observed (Fig. 2a).

### Medication

Figure 2b–d illustrates the use of asthma medication in the two groups. The cumulative daily ICS dose was calculated by summing the daily dose of budesonide (or

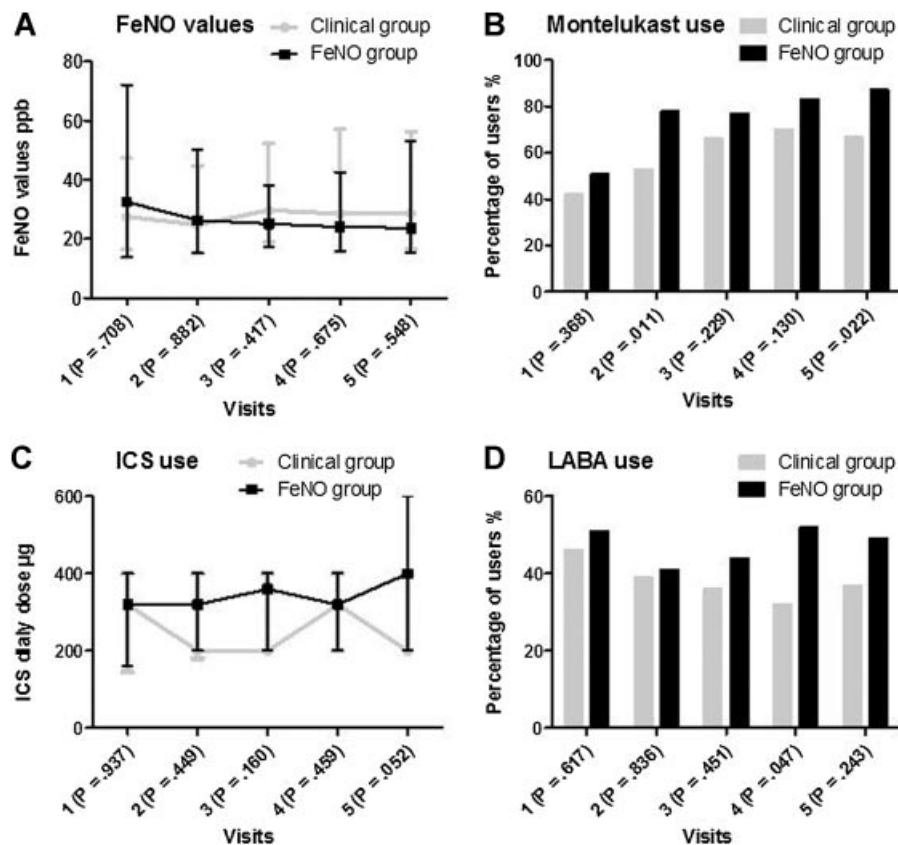


**Fig. 2.** FeNO values and medication use. Graphs A (FeNO values) and C (ICS use during the three preceding months) plot median values and interquartile ranges (Mann–Whitney test). Graphs B (LTRA use) and D (LABA use) illustrate the percentages of medication users at each visit (Chi-square-test). P-values are displayed under the X-axis.

equivalent) of visits 1–5. No differences were detected regarding the median daily ICS doses at all visits or the median cumulative daily ICS doses (interquartile range) between the clinical and FeNO groups: 1,200  $\mu\text{g}$  (675–1,600) and 1,280  $\mu\text{g}$  (800–1,800), respectively. However, in the FeNO group an increase of the ICS dose was observed between visits 1 and 5 (Wilcoxon Signed Ranks test,  $P = 0.013$ ); in the clinical group, no difference was seen between visits 1 and 5 ( $P = 0.649$ ). Subsequently, the change in daily ICS dose after 52 weeks of study follow-up was calculated by subtracting the daily ICS dose at visit 1 from the dose at visit 5. A median change of ICS dose (interquartile range) of 0  $\mu\text{g}$  (–200, +80) in the clinical group was demonstrated, compared with a change of +100  $\mu\text{g}$  (0, +400) in the FeNO group ( $P = 0.016$ ). Regarding LABA and LTRA use, the months of consumption were accounted during the 52-week follow-up. No difference was noted in the median (interquartile range) number of months of LABA use: 0 (0–9.8) in the clinical group and 6 (0–12) in the FeNO group. In the FeNO group, more months of leukotriene receptor antagonist use (median (interquartile range)) were observed: 12 (9–12) months, compared with 9 (3–12) months in the clinical group ( $P = 0.019$ ).

### The Discordance:Concordance Ratio

As a post-hoc analysis, we calculated the discordance: concordance ratio (D:C ratio), as an additional metric for FeNO trials.<sup>20,21</sup> The D:C ratio assesses the agreement between the study algorithm decisions. For every follow-up occasion, the therapy decision advised according to the applied algorithm can be compared with the decision that would have been advised if the patient had been allocated to the other algorithm. If both algorithms provide identical treatment recommendations for given decision points, it remains unlikely that a reliable difference in outcome parameters between the algorithms can be detected. The D:C ratio is calculated by dividing the number of times a decision is taken and the algorithms disagree, by the number of times the algorithms agree. In our trial, the decision-option “consider” complicated the calculation of the D:C ratio (see Table 1). Therefore, only those decision points during the trial were included if hypothetical comparison between the different algorithms was possible. After examining 74% of all decision points, we calculated a D:C ratio of 1.05.

### DISCUSSION

In our trial, the implementation of FeNO measurements in childhood allergic asthma management resulted in fewer exacerbations over a 52-week follow-up. Moreover, fewer children in the FeNO group experienced one or more exacerbations. However, the FeNO strategy did not alter the proportion of symptom-free days. Therapy adjustments

depending on FeNO measurements augmented the daily ICS doses between visits 1 and 5 and increased LTRA use.

A recent meta-analysis including six randomized controlled FeNO trials in children, adolescents and adults concluded that measuring FeNO to tailor the ICS dose cannot be routinely recommended for clinical practice at this stage.<sup>8</sup> Indeed, the results of these trials regarding the improvement of asthma control and in particular regarding the reduction of exacerbations have been equivocal.<sup>21</sup> However, these results must be seen in the light of several complex design issues. A substantial degree of methodological heterogeneity exists between these studies, namely regarding the definition of an exacerbation, the patient selection, the duration of the trial, algorithms for treatment adjustment and FeNO cut-off values.<sup>8</sup> Recently, the specific methodological design features of asthma treatment algorithm studies examining add-on FeNO were summarized.<sup>20,21</sup> The recommendation was to conduct multicenter, double-blind, parallel-group effectiveness trials to avoid additional bias, particularly evaluating asthma exacerbations as the most relevant primary outcome. Since well- and easily-controlled treatment-compliant patients experience few exacerbations and will probably not benefit much from additional FeNO measurement, it is additionally recommended to recruit a broad population in terms of asthma control, severity and level of therapy. Our trial was designed before these insights were published. Nevertheless, we came very close to these guidelines as we performed a multicentre, single-blind, parallel-group, randomized controlled trial in a moderately broad population regarding severity and level of therapy and focused on childhood allergic asthma.

Moreover, Gibson, Barnes and colleagues stress the need for an additional metric to assess the degree of agreement between the study algorithm decisions: the D:C ratio.<sup>20,21</sup> A low D:C ratio, between 0 and 1, could explain inconclusive study results, while a ratio score above 1 could indicate a potentially effective algorithm. We obtained a D:C ratio of 1.05, which indicates a potentially effective algorithm.

For the assessment of symptom-free days as the primary outcome, a surrogate marker of asthma control, we had to rely on a series of subjective questions on a diary card. Only 84% of participants succeeded in providing data from >310 days during the 52-week follow-up. The obtained patient-reported results must be interpreted in light of these limitations.

Further, we examined the occurrence of asthma exacerbations as a secondary outcome parameter, which was also suggested by the novel recommendations. Mild to severe exacerbations affect the patient's quality of life and daily activities and are therefore of interest as a main outcome parameter.<sup>8</sup> Exacerbations can be rare events and this might explain why the reduction in exacerbations in

our trial did not translate into significantly more symptom-free days. Consequently, an important limiting factor in order to capture asthma exacerbations is the duration of the trial. A limited follow-up period could hinder a truthful assessment of health benefits due to an intervention in the management of a chronic, highly variable illness such as asthma. The results of previous trials may not reflect the outcomes observed over a longer time period and can lead to biased or misleading conclusions about long-term effects.<sup>22,23</sup> We conducted a prospective study with a 52-week monitoring, comparable to three previous trials.<sup>19,24,25</sup> Pijnenburg and colleagues reported a non-significant difference in the occurrence of exacerbations: eight severe exacerbations in seven patients in the FeNO group versus eighteen exacerbations in ten patients in the clinical group.<sup>19</sup> The open label study of Verini and colleagues on the other hand showed a significant reduction of mild to severe asthma exacerbations.<sup>24</sup> Similarly, we revealed a significant reduction of asthma exacerbations in the FeNO group. These exacerbations were probably rather mild, since only three oral corticosteroid courses were prescribed in the FeNO group. Interestingly, our multiple logistic regression analysis confirmed that this reduction was the result of the implementation of FeNO measurements in the treatment algorithm. Moreover, the logistic regression analysis suggested an additional risk factor for the occurrence of asthma exacerbations: concurrent rhinitis, which is a known predisposing factor in the development of symptoms and asthma exacerbations.<sup>1</sup> The association between higher LTRA use and the occurrence of exacerbations over 52 weeks is probably more indirect, indicating that patients receiving more LTRA are also exposed to a higher exacerbation risk.

Administration of ICS forms the keystone of anti-inflammatory treatment. However, LTRA also deserves a place as an alternative or add-on option in the management of allergic childhood asthma, reducing the FeNO levels.<sup>1,12</sup> LTRA produces clinically relevant improvements in asthma and allergic rhinitis-related parameters, including exercise-induced symptoms, quality of life and the number of asthma exacerbations.<sup>1,26</sup> A recently published similar trial primarily focused on ICS titration and could not demonstrate a reduction in exacerbations.<sup>25</sup> Therefore, our algorithms contained the adjustment of ICS and LABA, but also of LTRA. Consequently, our study exhibited an increase in LTRA use in both groups, where children in the FeNO group received significantly more LTRA. In contrast, the median daily dose of ICS at each visit and the cumulative steroid dose did not differ between the two groups. Nevertheless, a trend toward higher doses of ICS in the FeNO group was translated into an increase of ICS doses in the FeNO group between visits 1 and 5. This might be related to our study design, in

which the decision arms contain multiple occasions to add LTRA or ICS. It could also be argued that the relatively lower median daily steroid doses and lower LTRA use in the clinical group indicate underdosing in some or inadequate treatment in others, resulting in more exacerbations. Nevertheless, looking at these results from an alternative point of view might indicate overtreatment in some participants in the FeNO group.

FeNO values in this trial did not differ between groups and did not change between visits 1 and 5. Although a trend toward decreased FeNO values was observed in the FeNO group, significance was not reached, probably due to the wide range of FeNO values. In our trial, the use of 20 ppb as the FeNO cut-off point was derived from previous data regarding upper limits of population-based normal values.<sup>15,16</sup> In other FeNO trials, cut-off values ranged from 12 up to 40 ppb.<sup>19,22–25,27</sup> Although reduction of FeNO levels to “normal” might seem desirable, a growing body of evidence implies that cut-off points should be based on FeNO levels characteristic of the population of interest instead.<sup>21</sup> For instance, the cut-off points for well-controlled asthma are different from, and might be higher than, FeNO values in normal subjects. In our trial, asthma control without exacerbations was also achieved in patients with FeNO levels above 20 ppb. Therefore, it has been proposed to include more than one cut-off point, for example separate cut-off points for increasing and decreasing anti-inflammatory treatment.<sup>20,21</sup> In this way, a decrease of medication could depend on the range of FeNO values found in patients with well-controlled asthma who remain stable after therapy reduction. On the other hand, FeNO values recorded in uncontrolled asthmatic patients at risk of adverse events could be used to define an appropriate cut-off point for an increase in medication.<sup>20</sup> Furthermore, substantial inter-individual variability of FeNO levels among clinically stable patients and variation of FeNO values by age have been identified.<sup>15,21</sup> For this reason, the percentage of change from baseline or from one’s personal best FeNO value could additionally be evaluated in order to increase or decrease medication.

We demonstrated that incorporating FeNO measurement in childhood asthma management was helpful in reducing asthma exacerbations and unscheduled contacts. The proportion of symptom-free days as a subjective outcome measure of asthma control did not differ between groups. Furthermore, an increase of LTRA use and ICS doses was detected in the FeNO group. We suggest exploration in greater depth of the modalities of FeNO cut-off values needed in the treatment algorithms and focusing further research on the occurrence of asthma exacerbations as the most relevant primary outcome. In accordance with recent study design recommendations, we strongly recommend further evaluation of FeNO measurements in double-blind, parallel-group, multicenter, randomized

controlled trials in broad populations in terms of asthma severity, control and level of asthma therapy.

## ACKNOWLEDGMENTS

We thank the children and parents for their participation in this trial. We address our gratitude to the Antwerp Provincial Institute for Hygiene and research nurse Danielle Stappers and the medical students Karen Van der Gucht, Jolien Van Herbruggen and Ellen Vancamp for their assistance in the data management. We are grateful to professor Kjell Alving, executive director of scientific and medical affairs of Aerocrine, Sweden for his helpful comments and to Kristien Wouters, PhD in medical statistics for her support in the statistical analyses.

## REFERENCES

1. From the Global Strategy for Asthma Management and Prevention, Global Initiative for Asthma (GINA) 2006. Available from: <http://www.ginasthma.org/>
2. Strunk RC, Szeffer SJ, Phillips BR, Zeiger RS, Chinchilli VM, Larsen G, Hodgdon K, Morgan W, Sorkness CA, Lemanske RF Jr. Relationship of exhaled nitric oxide to clinical and inflammatory markers of persistent asthma in children. *J Allergy Clin Immunol* 2003;112:883–892.
3. Tillie-Leblond I, Montani D, Crestani B, de Blic J, Humbert M, Tunon-de-Lara M, Magnan A, Roche N, Ostinelli J, Chanez P. Relation between inflammation and symptoms in asthma. *Allergy* 2009;64:354–367.
4. Sont JK, Han J, van Krieken JM, Evertse CE, Hooijer R, Willems LN, Sterk PJ. Relationship between the inflammatory infiltrate in bronchial biopsy specimens and clinical severity of asthma in patients treated with inhaled steroids. *Thorax* 1996;51:496–502.
5. van den Toorn LM, Overbeek SE, de Jongste JC, Leman K, Hoogsteden HC, Prins JB. Airway inflammation is present during clinical remission of atopic asthma. *Am J Respir Crit Care Med* 2001;164:2107–2113.
6. Prescott SL. The development of respiratory inflammation in children. *Paediatr Respir Rev* 2006;7:89–96.
7. Pijnenburg MW, Hofhuis W, Hop WC, De Jongste JC. Exhaled nitric oxide predicts asthma relapse in children with clinical asthma remission. *Thorax* 2005;60:215–218.
8. Petsky HL, Cates CJ, Lasserson TJ, Li AM, Turner C, Kynaston JA, Chang AB. A systematic review and meta-analysis: tailoring asthma treatment on eosinophilic markers (exhaled nitric oxide or sputum eosinophils). *Thorax* 2012;67:199–208.
9. Nelson BV, Sears S, Woods J, Ling CY, Hunt J, Clapper LM, Gaston B. Exhaled nitric oxide as a marker for childhood asthma. *J Pediatr* 1997;130:423–427.
10. Alving K, Malinowski A. Basic aspects of exhaled nitric oxide. In: Horvath I, de Jongste JC, editors. *Exhaled Biomarkers, European Respiratory Monograph*. Lausanne: European Respiratory Society; 2010. pp 1–31.
11. 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.
12. Verini M, Peroni DG, Piacentini GL, Nicodemo A, Rossi N, Bodini A, Chiarelli F, Boner AL. Comparison of add-on therapy to inhaled fluticasone propionate in children with asthma: residual volume and exhaled nitric oxide as outcome measures. *Allergy Asthma Proc* 2007;28:691–694.
13. Harkins MS, Fiato KL, Iwamoto GK. Exhaled nitric oxide predicts asthma exacerbation. *J Asthma* 2004;41:471–476.
14. Khalili B, Boggs PB, Bahna SL. Reliability of a new hand-held device for the measurement of exhaled nitric oxide. *Allergy* 2007;62:1171–1174.
15. Buchvald F, Baraldi E, Carraro S, Gaston B, De Jongste J, Pijnenburg MW, Silkoff PE, Bisgaard H. Measurements of exhaled nitric oxide in healthy subjects age 4 to 17 years. *J Allergy Clin Immunol* 2005;115:1130–1136.
16. Kharitonov SA, Gonio F, Kelly C, Meah S, Barnes PJ. Reproducibility of exhaled nitric oxide measurements in healthy and asthmatic adults and children. *Eur Respir J* 2003;21:433–438.
17. 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 J Respir Crit Care Med* 2005;171:912–930.
18. Liu AH, Zeiger R, Sorkness C, Mahr T, Ostrom N, Burgess S, Rosenzweig JC, Manjunath R. Development and cross-sectional validation of the childhood asthma control test. *J Allergy Clin Immunol* 2007;119:817–825.
19. Pijnenburg MW, Bakker EM, Hop WC, De Jongste JC. Titrating steroids on exhaled nitric oxide in children with asthma: a randomized controlled trial. *Am J Respir Crit Care Med* 2005;172:831–836.
20. Gibson PG. Using fractional exhaled nitric oxide to guide asthma therapy: design and methodological issues for Asthma Treatment Algorithm studies. *Clin Exp Allergy* 2009;39:478–490.
21. Barnes PJ, Dweik RA, Gelb AF, Gibson PG, George SC, Grasemann H, Pavord ID, Ratjen F, Silkoff PE, Taylor DR, Zamel N. Exhaled nitric oxide in pulmonary diseases: a comprehensive review. *Chest* 2010;138:682–692.
22. Fritsch M, Uxa S, Horak FJr, Putschogl B, Dehlink E, Szepfalusi Z, Frischer T. Exhaled nitric oxide in the management of childhood asthma: a prospective 6-months study. *Pediatr Pulmonol* 2006;41:855–862.
23. de Jongste JC, Carraro S, Hop WC, Study CHARISM Group, Baraldi E. Daily telemonitoring of exhaled nitric oxide and symptoms in the treatment of childhood asthma. *Am J Respir Crit Care Med* 2009;179:93–97.
24. Verini M, Consilvio NP, Di Pillo S, Cingolani A, Spagnuolo C, Rapino D, Scaparrotta A, Chiarelli F. FeNO as a marker of airways inflammation: the possible implications in childhood asthma management. *J Allergy (Cairo)* 2010;2010:1–7.
25. Pike K, Selby A, Price S, Warner J, Connett G, Legg J, Lucas JS, Peters S, Buckley H, Magier K, Foote K, Drew K, Morris R, Lancaster N, Roberts G. Exhaled nitric oxide monitoring does not reduce exacerbation frequency or inhaled corticosteroid dose in paediatric asthma: a randomised controlled trial. *Clin Respir J* 2012 [Epub ahead of print].
26. Diamant Z, Mantzouranis E, Bjerner L. Montelukast in the treatment of asthma and beyond. *Expert Rev Clin Immunol* 2009;5:639–658.
27. Szeffer SJ, Mitchell H, Sorkness CA, Gergen PJ, O'Connor GT, Morgan WJ, Kattan M, Pongracic JA, Teach SJ, Bloomberg GR, Eggleston PA, Gruchalla RS, Kerckmar CM, Liu AH, Wildfire JJ, Curry MD, Busse WW. Management of asthma based on exhaled nitric oxide in addition to guideline-based treatment for inner-city adolescents and young adults: a randomised controlled trial. *Lancet* 2008;372:1065–1072.

## SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher's web-site.

*E-table 1 FEV<sub>1</sub> measurements*

|                            | Visit 1                    | Visit 2                     | Visit 3                    | Visit 4                    | Visit 5                    | Visit 5 compared<br>with visit 1 <sup>b</sup> |
|----------------------------|----------------------------|-----------------------------|----------------------------|----------------------------|----------------------------|-----------------------------------------------|
| <b>Clinical group</b>      | 89.0<br>(16.2, 53.0-123.3) | 90.7 (13.2, 61.7-<br>126.0) | 88.7<br>(15.4, 41.0-129.0) | 91.3<br>(13.5, 61.4-134.4) | 91.2<br>(12.3, 59.8-117.6) | <b>0.40</b>                                   |
| <b>FeNO group</b>          | 92.9<br>(12.2, 67.4-125.5) | 92.2.<br>(14.1, 60.9-117.0) | 92.4<br>(13.6, 52.4-120.9) | 93.1<br>(13.9, 66.8-126.3) | 93.9<br>(15.5, 51.1-147.1) | <b>0.70</b>                                   |
| <b>P-value<sup>a</sup></b> | <b>0.18</b>                | <b>0.60</b>                 | <b>0.22</b>                | <b>0.51</b>                | <b>0.36</b>                |                                               |

E-table 1. FEV<sub>1</sub>, (mean % predicted, SD, range: minimum-maximum) values are reported. a Student's *t* test, b Paired samples *t* test

*E-table 2* **FEV<sub>1</sub> measurements post bronchodilator**

|                            | Visit 1                  | Visit 5                  | Visit 5 compared with visit 1 <sup>b</sup> |
|----------------------------|--------------------------|--------------------------|--------------------------------------------|
| <b>Clinical group</b>      | 96.7 (13.9, 67.0-127.8)  | 99.5 (10.2, 68.6-122.6)  | <b>0.19</b>                                |
| <b>FeNO group</b>          | 101.3 (11.8, 78.6-144.5) | 101.6 (10.5, 80.2-120.3) | <b>0.95</b>                                |
| <b>P-value<sup>a</sup></b> | <b>0.08</b>              | <b>0.33</b>              |                                            |

E-table 2. FEV<sub>1</sub> post bronchodilator,(mean % predicted, SD, range: minimum-maximum) values are reported. a Student's *t* test, b Paired samples *t* test

*E-table 3* **Reversibility of FEV<sub>1</sub> measurements**

|                            | <b>Visit 1</b>     | <b>Visit 5</b>      | <b>Visit 5 compared with visit 1<sup>b</sup></b> |
|----------------------------|--------------------|---------------------|--------------------------------------------------|
| <b>Clinical group</b>      | +6.9<br>(1.8-16.0) | +9.00<br>(4.4-12.7) | <b>0.73</b>                                      |
| <b>FeNO group</b>          | +8.4<br>(3.3-15.0) | +7.91<br>(4.6-12.1) | <b>0.50</b>                                      |
| <b>P-value<sup>a</sup></b> | <b>0.64</b>        | <b>0.73</b>         |                                                  |

E-table 3. Reversibility (median% change, interquartile range) values are reported. a Mann-Whitney test, b Wilcoxon Signed Ranks test