

Phenotyping patients with chronic cough: Evaluating the ability to predict the response to anti-inflammatory therapy



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ABSTRACT

Background: Whether the fraction of exhaled nitric oxide (FeNO) measurement can predict the response to anti-inflammatory treatment in chronic cough is unknown.

Objective: To explore whether the effectiveness of treatment with 10 mg of montelukast or 20 mg of prednisolone in patients with chronic cough is predicted by FeNO level.

Methods: In this randomized, open-label, controlled pilot study conducted in the Clinical Trial Unit in Castle Hill Hospital in the United Kingdom, 50 nonsmoking patients with a cough that lasted more than 8 weeks were sequentially enrolled in the study. Thirty patients with high FeNO levels (≥ 30 ppb) were randomized in a 1:1 ratio to receive 10 mg of montelukast or 20 mg of prednisolone for 2 weeks followed by 10 mg of montelukast for 2 weeks. Twenty patients with a low FeNO level (≤ 20 ppb) received 10 mg of montelukast. The primary objective was to determine the effectiveness of treatment on 24-hour cough counts.

Results: The 24-hour cough counts decreased in both groups by approximately 50% ($P < .005$), indicating that FeNO did not predict treatment response. However, it was a good marker for eosinophilic inflammation with a high degree of correlation with blood and sputum eosinophilia ($P < .001$).

Conclusion: These results suggest that prior investigation may not predict response to anti-inflammatory treatment, which may be consequent on localized leukotriene-mediated inflammation.

Trial Registration: [ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT02479074) Identifier: NCT02479074.

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Introduction

Airway eosinophilic inflammation is a characteristic feature of classic childhood atopic asthma.^{1,2} The fraction of exhaled nitric oxide (FeNO) is an established biomarker of this inflammation.³ A proportion of patients with isolated chronic cough also have eosinophilic inflammation but present without bronchoconstriction, conditions termed *cough variant asthma* or *eosinophilic bronchitis*.^{4–9} Treatment with anti-inflammatory agents, such as prednisolone and montelukast, may be a successful strategy in these patients.^{10–12} However, whether an estimation of FeNO value can help guide therapy is unknown. We hypothesized that FeNO, measured at presentation, should allow the clinician to target anti-inflammatory therapy administered as first-line treatment. We performed a randomized clinical study to examine the outcome of anti-inflammatory

therapy with the leukotriene antagonist montelukast or oral prednisolone in patients presenting to a specialist cough clinic.

Methods

Study Design

This was a randomized, open-label, controlled pilot study to examine the outcome of anti-inflammatory therapy with an associated elevated FeNO in chronic cough. Patients presenting a high FeNO measurement (arbitrarily defined as FeNO ≥ 30 ppb)^{13,14} were compared with a contemporaneously recruited group with low FeNO measurements (defined as ≤ 20 ppb).

The primary objective of this study was to determine the efficacy of treatment on 24-hour cough counts. Subjective measures of cough were compared using the Hull Airways Reflux Questionnaire (HARQ) and Leicester Cough Questionnaire (LCQ). Changes in the sputum inflammatory cells were also evaluated. Detailed methods are available in the online supplement.

This study consisted of 5 visits. In the screening visit, informed consent was obtained before baseline assessments. After baseline assessments, eligible individuals with FeNO levels of 30 ppb or

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greater were randomized in a 1:1 ratio to receive 2 weeks of 10-mg montelukast film-coated tablets or 20-mg prednisolone tablets followed by 10-mg montelukast film-coated tablets for the subsequent 2 weeks in both arms. The effect of the prednisolone trial was used as a positive control to determine the maximum effect of anti-inflammatory therapy. Allocation was performed by the Research and Development Department of NHS Trust based on a balanced block randomization scheme, which was prepared using a computerized system-sealed envelope. After baseline assessments, individuals with FeNO levels of 20 ppb or less received 10-mg montelukast film-coated tablets for 4 weeks.

In the first visit, the following assessments and procedures were performed; FeNO measurement, spirometry, sputum collection, full blood cell count, 24-hour cough count, HARQ, and LCQ. After a mean (SD) of 13 (2) and 27 (2) days in the second and fourth visits, the Hull Automated Cough Counter (HACC) was applied to measure patients' cough frequency for 24 hours. The next day after these visits in the third and fifth visits the following procedures were repeated: FeNO measurement, spirometry, sputum collection, HARQ, and LCQ. At the end of the study, all patients were reviewed by a chronic cough registrar or consultant as per the standard care as a clinic patient.

The study was approved by the National Research Ethics Service Committee North East-Tyne & Wear South (EudraCT 2015-001736-38) and registered with [ClinicalTrials.gov](https://www.clinicaltrials.gov) (NCT02479074).

Patient Selection

Patients were recruited sequentially from the Hull Cough Clinic in Castle Hill Hospital in the United Kingdom. We planned to recruit

40 patients with chronic cough with an FeNO of 30 ppb or greater. However, patients with high FeNO levels represented only 10% of the clinic population at the time of the study, and because of slow recruitment it was decided to stop the study after enrolling 30 patients. Twenty patients with chronic cough and an FeNO of 20 ppb or less were enrolled (Fig 1).

Patients with a history of chronic cough of at least 8 weeks' duration were included in the study. Patients with the following criteria were excluded from the study: current diagnosis of classic asthma as demonstrated by variable airflow obstruction, any significant concomitant disease, a lower respiratory tract infection in the last 4 weeks, use of angiotensin-converting enzyme inhibitors, and current smoking. The concomitant use of inhaled corticosteroids or bronchodilators was allowed provided dosing was stable for at least 4 weeks before enrollment. (None of the patients in the low-FeNO group were using inhalers.)

Procedures

After randomization, patients in the first treatment arm with high FeNO levels received 4 weeks of treatment with 10-mg montelukast film-coated tablets to take 1 tablet per day at night. Patients in the second treatment arm received 5-mg prednisolone tablets to take 4 tablets per day (20 mg) for 2 weeks in the morning followed by 10-mg montelukast film-coated tablets to take 1 tablet at night for an additional 2 weeks. The low-FeNO group received 4 weeks of treatment with 10-mg montelukast film-coated tablets to take 1 tablet per day at night.

The medicines were prescribed by a clinician in an open-label fashion at the first visit 1 (day 1) and to the second arm treatment

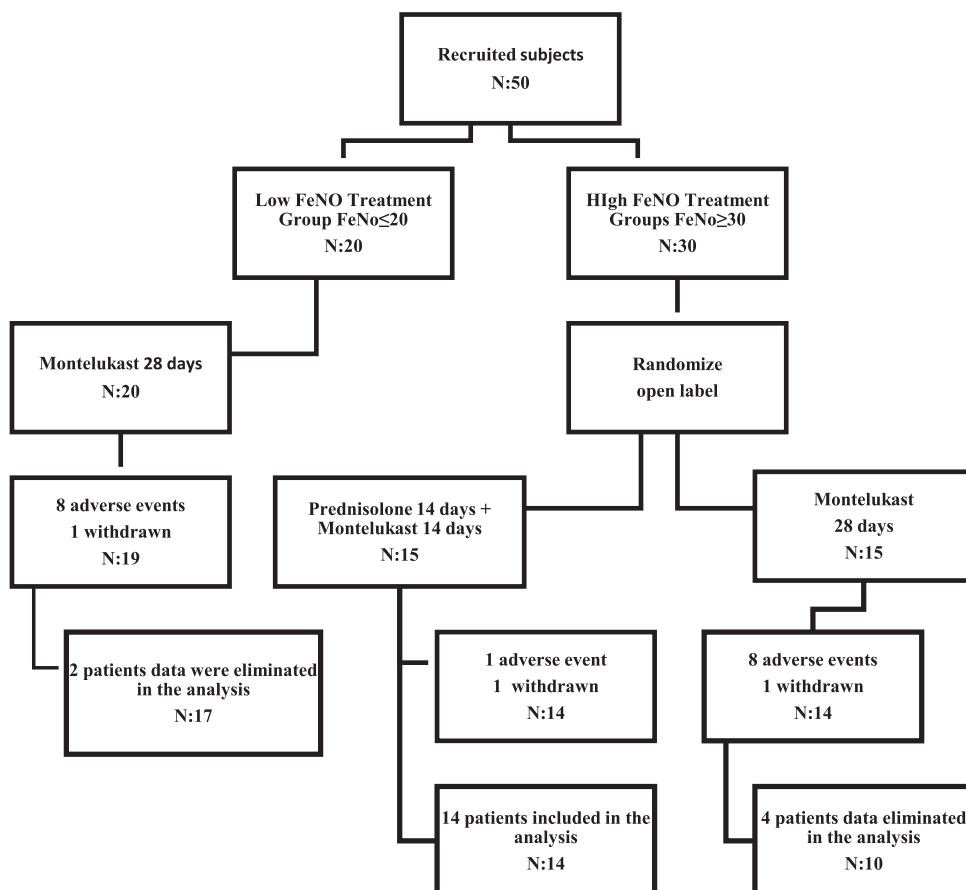


Figure 1. Consort flow diagram. FeNO indicates fraction of exhaled nitric oxide.

group at the first visit (day 1) and third visit (day 14). Study medications were stored and dispensed by the trial site's pharmacy department in accordance with good clinical practice. Prednisolone was packaged in a blister pack, propylene container, or glass bottle. Montelukast was packaged in blister foil.

FeNO Measurement

FeNO was measured (NIOX VERO machine, Aerocrine Ltd, Morrisville, North Carolina) at an expiratory flow rate of 50 mL/s in visits 1, 3, and 5, according to the 2005 American Thoracic Society (ATS)/European Respiratory Society (ERS) recommendations.¹⁵ A calibrated electrochemical sensor analyses the last 3 seconds of the 10-second exhalation to indicate results in parts per billion with a measurement range of 5 to 300 ppb. FeNO less than 25 ppb in adults is less likely to indicate eosinophilic inflammation and response to corticosteroids.¹⁶

Lung Function

A pneumotach within a spirometer was used to measure lung function according to the specifications and performance criteria recommended by the ATS/ERS standardization of spirometry.¹⁷

24-Hour Cough Count

The HACC and Leicester Cough Monitor (LCM) software was used to measure the cough frequency during a 24-hour period. The automated assessment of cough is valid, reliable, and highly reproducible^{18,19} and significantly correlated with subjective assessment of cough and cough reflex sensitivity.²⁰

Sputum Induction and Process

Sputum samples were collected by applying different techniques, such as spontaneous expectoration or sputum induction. An ultrasonic nebulizer (DeVilbiss UltraNeb ultrasonic nebulizer) with a mean output of 1 mL/min was used to generate aerosols with a dose of approximately 5 to 7 mL per inhalation to collect induced sputum.³ The device was set according to the standard operating procedure of clinical trial unit CTU101099. The standard operating procedure of clinical trial unit SOPCTU100210 has been used to process the sputum samples, although some minor alterations have been applied.

In this study, sputum samples were scheduled to be collected from each participant at visits 1, 3, and 5. However, it was challenging to collect sputum samples, especially after treatment, in visits 3 and 5. In some cases, the produced samples were not cellular enough to process. In this analysis, we have included data from each patient who was able to produce a sample at baseline and one from the last or third visit. Overall, there were 25 patients who had 2 samples before and after treatment (high FeNO first arm = 7, high FeNO second arm = 8, and low FeNO = 10).

HARQ and LCQ

HARQ and LCQ were used as subjective measures of cough. HARQ is a 14-point questionnaire; each question independently tests for the cough hypersensitivity syndrome on a scale of 0 to 5 (0, no problem; 5, severe/frequent problems), with the total score varying from 0 to 70 points and the upper limit of normal being 13 of 70. The LCQ contains 19 questions to assess 3 domains of physical, psychological, and social with a 7-point Likert response scale, ranging from 1 for all of the time to 7 for none of the time. The score varied from 3 to 21, with a higher score indicating better quality of life and a change of 2.56 in total LCQ score more likely to be significant.²¹

Statistical Analysis

Because this is a feasibility study to generate essential data to inform the power analysis of a larger scale, sample size calculation would not be necessary. Patients' ages and sex, FeNO, 24-hour cough count, LCQ and HARQ responses, spirometry measurement, sputum eosinophilic count, and blood eosinophilic (B-Eos) count were expressed as mean (SD) using SPSS statistical software (SPSS Inc, Chicago, Illinois).

Repeated-measures analysis of covariance was used to compare changes in the number of coughs in 24 hours, sputum eosinophil cell count, spirometry measurements, and HARQ and LCQ scores at baseline and after 2 and 4 weeks of treatment between high-FeNO treatment groups and low-FeNO treatment group.

Analysis of covariance was used to compare changes in the FeNO value at baseline and after 2 and 4 weeks of treatment among the 3 treatment groups to assess whether the efficiency of FeNO will help to predict therapeutic response to anti-inflammatory medication in cough. $P < .05$ was considered significant.

Results

During a 15-month period, 50 patients were recruited into the study: 15 patients in each high-FeNO group and 20 patients in the low-FeNO group. Three patients were withdrawn from the study, 1 because of an error in the randomization (high-FeNO second-arm group) and 2 (1 from the high-FeNO first-arm group and 1 from the low-FeNO group) because of severe cough associated with montelukast. Thus, 47 patients completed the study: 28 in the high-FeNO group and 19 in the low-FeNO group. No serious adverse events occurred; however, 17 patients reported minor events during the study of whom 5 experienced upper respiratory tract infection symptoms. Because upper respiratory tract infection affects FeNO and 24-hour cough count, the data from these patients were not used in the analysis. Thus, data from a total of 41 patients were included in the analysis (high FeNO first arm = 10, high FeNO second arm = 14, and low FeNO = 17).

Mean (SD) age of the patients studied was 62 (9.5) years (range, 45–82 years). A total of 32 patients (65%) were female, whereas only 17 patients (35%) were male. Mean (SD) forced expiratory volume in 1 second and forced vital capacity were 105% (20%) and 115% (21%) of predicted, respectively. There was no significant change in lung function during the study. At baseline, the mean (SD) B-Eos count in the high-FeNO group was $0.34 (0.2) \times 10^9/L$ (range, $0.1-1 \times 10^9/L$), with 50% of the patients having a B-Eos count greater than $0.3 \times 10^9/L$. In the low-FeNO group, the mean (SD) B-Eos count was $0.16 (0.1) \times 10^9/L$ (range, $0.05-0.5 \times 10^9/L$). In this group, a single patient had a B-Eos count greater than 0.3 to $0.56 \times 10^9/L$. There was a marked difference in the sex split, with equal numbers of male and female patients in the high-FeNO group, whereas the low-FeNO group almost entirely consisted of women (Table 1). None of the patients in the low-FeNO group were using inhaled corticosteroids or bronchodilators. Only 4 patients in the high-FeNO group were using corticosteroid inhalers.

Table 1
Age and Sex of the Study Patients

Treatment Group	No. of Patients	Sex, No. (%)		Mean Age, y
		Male	Female	
High FeNO arm 1	15	8	7	59
High FeNO arm 2	14	7	7	65
Low FeNO	20	2	18	62
Total	49 (100)	17 (35)	32 (65)	62

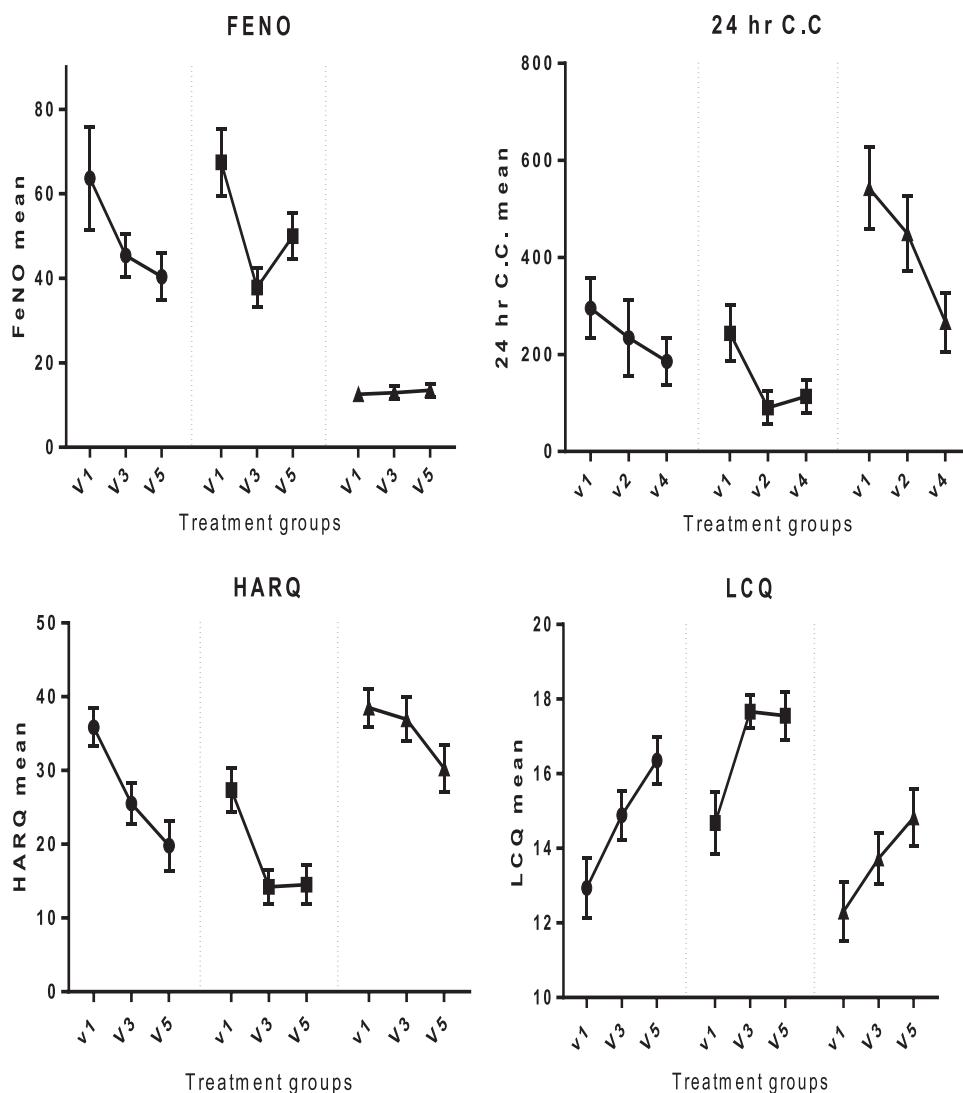


Figure 2. Measurements of fraction of exhaled nitric oxide (FeNO), 24-hour cough count, Hull Airways Reflux Questionnaire (HARQ), and Leicester Cough Questionnaire (LCQ) in 3 treatment groups during 3 visits (visits 1, 3, and 5). Error bars indicate SEM.

FeNO was measured with a NIOX VERO machine (Aerocrine Ltd) at an expiratory flow rate of 50 mL/s at visits 1, 3, and 5, according to the 2005 ATS/ERS recommendations.¹⁵ The mean (SD) FeNO in the montelukast-treated high-FeNO group was 56 (33) ppb at baseline. After 2 and 4 weeks treatment, this level decreased to 41 (16) ppb and 39 (17) ppb. In the high-FeNO prednisolone arm, the mean (SD) FeNO decreased from 67 (30) ppb to 38 (18) ppb after 2 weeks of treatment with prednisolone followed by a significant ($P < .05$) increase to 50 (20) ppb after 2 weeks of further treatment with montelukast (Fig 2). Thus, at the end of the study, there was a significant decrease in FeNO of approximately 30% in both high-FeNO treatment groups ($P < .005$). In the low-FeNO group, there was no significant ($P < .60$) change during the study: 12 (5) ppb at baseline, 13 (7) ppb at 2 weeks, and 14 (7) ppb at 4 weeks.

The HACC and LCM software was used to measure the cough frequency during a 24-hour period. The automated assessment of cough is a valid, reliable, and highly reproducible^{18,19} and significantly correlated with subjective assessment of cough and cough reflex sensitivity.²⁰ HACC device failure led to loss of data on 3 occasions. The predominantly female low-FeNO group had more than twice the number of coughs recorded. The 24-hour cough count decreased after 14 days and 28 days of treatment compared with

baseline in all 3 groups ($P < .005$) (Fig 2). In the low-FeNO group, the mean (SD) total 24-hour cough count was reduced from 566 (388) to 449 (315) and 265 (267) after 2 and 4 weeks of treatment with montelukast, representing a 53% overall reduction in recorded cough. In the high-FeNO groups, a similar trend can be seen. The mean (SD) number of coughs in 24 hours decreased 49% from 292 (158) to 173 (63) and 150 (104) with 4 weeks montelukast. Initial prednisolone therapy reduced the number of coughs from 237 (223) to 89 (132) but then significantly ($P < .05$) increased the number of coughs to 114 (122) when montelukast was substituted. Thus, both high-FeNO groups and the low-FeNO group cough improved by approximately 50% during the study (Fig 3).

In all treatment groups, there was a significant improvement in the HARQ score during the study ($P < .005$) (Fig 2). After 2 weeks of therapy, the mean (SD) low-FeNO group HARQ score decreased to 37 (13) and 31 (14) at the end of the study. In the high-FeNO group, montelukast decreased the mean (SD) HARQ score from 33 (5) to 23 (8) and 20 (11); in the prednisolone arm, scores almost halved from 27 (11) to 14 (9), and the score was 15 (10) after montelukast substitution.

Similar to the HARQ findings, there was a significant ($P < .005$) improvement in LCQ scores in all groups during the study. Patients

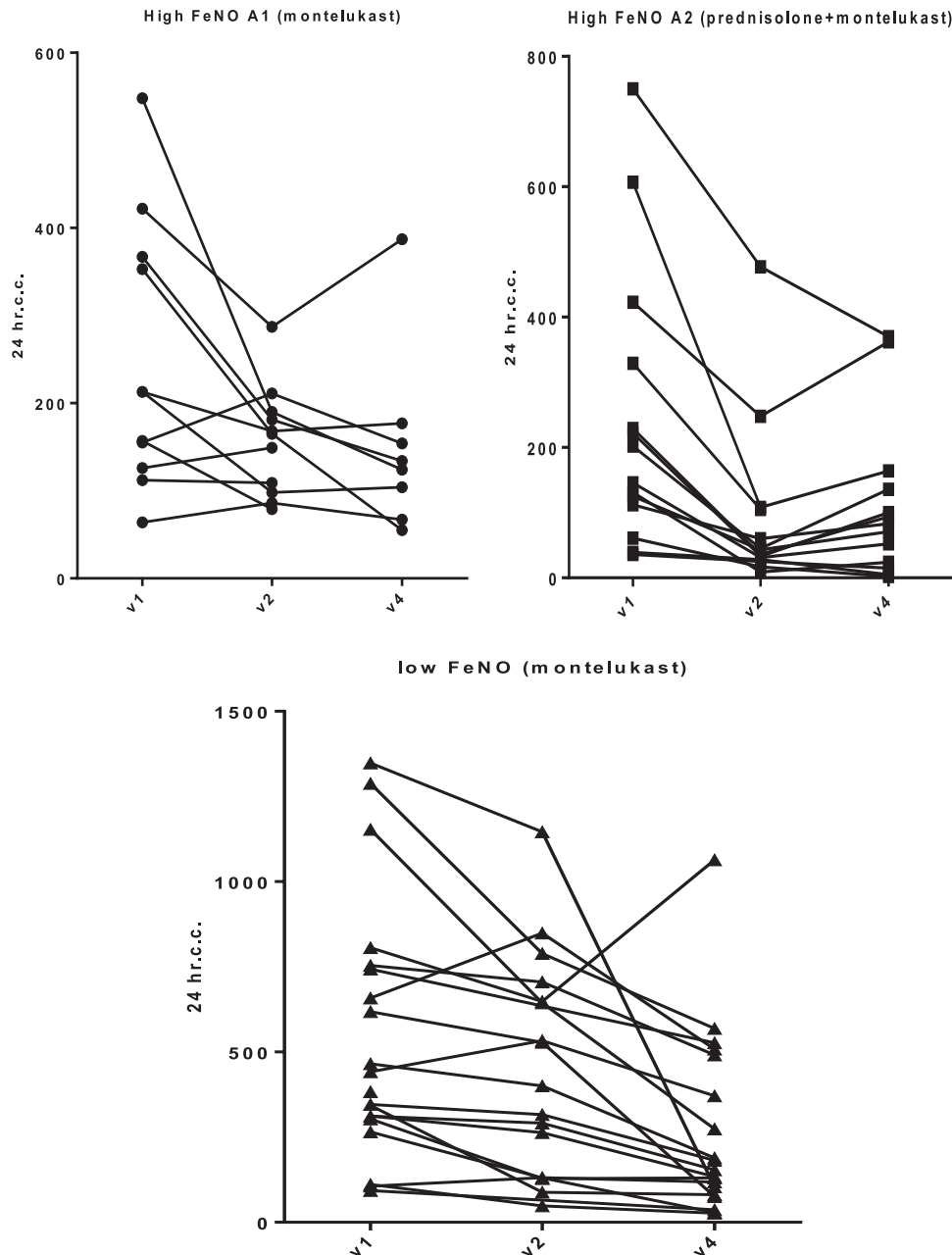


Figure 3. Changes in 24-hour cough counts in the individual patients in 3 treatment groups at 3 visits (visits 1, 3, and 5). FeNO indicates fraction of exhaled nitric oxide.

with low FeNO levels scored a mean (SD) of 12 (4) at baseline and 14 (3) and 15 (3), respectively, after 2 and 4 weeks of treatment. In the montelukast-treated high-FeNO group, the scores improved from 14 (3), 15 (2), and 16 (2); in the prednisolone arm, the scores also improved from 15 (3) to 18 (2), and this was maintained for the second treatment period (Fig 2).

In this study, sputum samples were scheduled to be collected from each participant at visits 1, 3, and 5. However, it was challenging to collect sputum samples, especially after treatment, at visits 3 and 5. In some cases, the produced samples were not cellular enough to process. In this analysis, we have included data from each patient who was able to produce a sample at baseline and one from the last or third visit. Overall, there were 25 patients who had 2 samples before and after treatment (high FeNO first arm = 7, high FeNO second arm = 8, and low FeNO = 10).

At baseline, a strong correlation was found between the sputum eosinophil count and FeNO value ($r = 0.65$, $P < .001$). The mean (SD) sputum eosinophil count in the high-FeNO group treated by montelukast significantly ($P < .05$) decreased from 11.7% (26%) to 1.8% (1.9%). With prednisolone, sputum eosinophil counts decreased from 8.6% (17%) to 2.6% (5.9%). Therefore, both treatment regimens had a similar effect on the eosinophil counts in the sputum. In the low-FeNO group, sputum eosinophilia was below 1% before and after treatment.

Discussion

In a few studies of chronic cough, the diagnostic value of FeNO measurement has been evaluated.^{13,14,22–24} In studies in which the FeNO value was specifically examined in differentiation of asthmatic

cough from nonasthmatic cough,^{13,14} a cut point of FeNO greater than ppb was suggested to be more likely to favor asthmatic cough. Others have reported that the FeNO value is significantly higher in cough variant asthma and eosinophilic bronchitis compared with atopic cough and upper airway cough syndrome.^{22–24} However, the value of FeNO to monitor therapeutic response to montelukast in chronic cough has not previously been examined.

There were several anticipated findings of this study. The hypothesis that FeNO could be used as a marker of eosinophilic inflammation in chronic cough was supported by our observation at baseline in the high-FeNO group of eosinophilia in both blood and sputum. Only a single patient in the low-FeNO group had a B-Eos count greater than $0.3 \times 10^9/L$. In recent years, there has been considerable controversy regarding the use of B-Eos counts and FeNO values in other airways disease.^{25–27} We consider that sampling error attributable to the variability of these measures may be responsible for some of this controversy.²⁸

Perhaps the most surprising aspect of our study is the response in the low-FeNO group to montelukast based on both objective and subjective measurements of cough. Montelukast was used as a control treatment in the low-FeNO group at the suggestion of the institutional review board in the anticipation of it having little or no effect in those without eosinophilic inflammation. However, we found a similar degree of response to therapy in both the low- and high-FeNO groups. This finding could infer that in our study population there was a large element of the placebo response but the markers of eosinophilia, B-Eos count, and sputum eosinophil count decreased in the high-FeNO group (as they do in allergic asthma), suggesting biological activity.

Montelukast has been repeatedly demonstrated to cause allergic inflammation in asthma²⁹ and more recently in eosinophilic cough.^{30,31} This activity is assumed to be attributable to blockade of leukotriene receptors.³² If montelukast is not simply a placebo, it suggests that the current markers of eosinophilic lung disease are insufficiently sensitive to pick up low levels of leukotriene activation in the low-FeNO group or that montelukast has its antitussive activity by an alternative mechanism.

In this study, both subjective and objective measures of coughs responded to therapy in a consistent fashion. These metrics do not measure the same domains of cough, with there being an approximately 60% correlation between these measures.²⁰ That we saw a similar degree of response in these diverse metrics suggests that the reduction in the cough was a real observation.

In patients with low FeNO levels, montelukast may have alternative modes of action other than to antagonize eosinophilic inflammation. Anti-inflammatory activity targeting the neuronal hypersensitivity in these patients has been suggested. Mast cells have an ability to interact with airway nerves.^{33,34} The response of patients with chronic cough to antagonists of neuronal receptors, such as the P2X3³⁵ and NK1,³⁶ is beginning to unravel the mechanism of cough hypersensitivity in humans. A localized mast cell and afferent neuron interaction may be relevant to the pathogenesis of cough in patients without systemic evidence of eosinophilia. Taylor-Clark et al³⁷ found that application of cysteinyl leukotrienes causes an increase in afferent neuronal excitability as measured by patch clamp and isolated single-fiber recordings, which was inhibited by cysteinyl leukotriene 1 antagonists.

Montelukast has previously been found to reduce subjective cough scores in nonasthmatic patients with chronic cough. A small study³⁰ also found that cough reflex sensitivity to capsaicin was decreased significantly, supporting the argument for modification of neuronal hypersensitivity. In an airway biopsy study in asthma, Ramsay et al³⁸ reported that treatment with montelukast reduced the number of not only eosinophils but also tissue mast cells. Therefore, montelukast may inhibit cough by blocking cysteinyl leukotrienes produced by mast cells, which activate

airway nerves, an observation previously made in cough variant asthma.³⁹

In conclusion, the results of this study suggest treatment of chronic cough with montelukast is not reliant on the demonstration of eosinophilic inflammation. A larger placebo-controlled study is required to confirm the degree of treatment response in the noneosinophilic population.

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