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Urinary Leukotriene E4 /Exhaled Nitric Oxide Ratio and Montelukast Response in Childhood Asthma

Nathan Rabinovitch, MD[1], **Nora J Graber, MS**[2], **Vernon M. Chinchilli, PhD**[2], **Christine A. Sorkness, PharmD**[3], **Robert S. Zeiger, MD, PhD**[4], **Robert C Strunk, MD**[5], **Leonard B. Bacharier**[5], **Fernando D. Martinez, MD**[6], and **Stanley J. Szefler, MD**[1] **for the Childhood Asthma Research and Education Network of the Heart, Lung and Blood Institute**

[1]Department of Pediatrics, National Jewish Health, Denver

[2]University of Colorado Denver, School of Medicine, Denver

[3]Department of Public Health Sciences, Pennsylvania State University, Hershey

[4]Schools of Pharmacy and Medicine and Public Health, University of Wisconsin, Madison

[5]Department of Pediatrics, University of California-San Diego; Department of Allergy, Kaiser Permanente San Diego

[6]Department of Pediatrics, Washington University School of Medicine, St. Louis, MO. Department of Pediatrics, University of Arizona, Phoenix, AZ

Abstract

Background—A subset of children with asthma respond better to leukotriene receptor antagonists (LTRA) than to inhaled corticosteroids (ICS). Information is needed to identify children with these preferential responses.

Objective—To determine whether the ratio of urinary leukotriene E_4 to fractional exhaled nitric oxide (LTE_4: FE_{NO}) delineates children with preferential responsiveness to montelukast (MT) compared to fluticasone propionate (FP) therapy.

Methods—Data from 318 children with mild to moderate asthma enrolled in 2 NHLBI CARE network studies (CLIC and PACT) were analyzed. The association between LTE_4: FE_{NO} ratios at baseline and improved lung function or asthma control days (ACDs) with MT and FP therapy was determined and phenotypic characteristics related to high ratios was assessed.

Results—LTE_4: FE_{NO} ratios were associated with a greater response to MT than FP therapy for forced expiratory volume in 1 second (FEV_1) measurements (2.1% increase per doubling of ratio, p=0.001) and for ACDs per week (0.3 increase, p= 0.009) in the CLIC study. In PACT, the ratio was associated with greater FEV_1 responsiveness to MT than FP therapy (0.6% increase, p= 0.03). In a combined study analysis, LTE_4: FE_{NO} ratios were associated with greater response to MT than FP therapy for FEV_1 (0.8% increase, p=0.0005) and ACDs (0.3 increase, p=0.008). Children with LTE_4: FE_{NO} ratios at or above the 75th percentile were likely (p<0.05) to be younger, female and exhibit lower levels of atopic markers and methacholine reactivity.

Reprint Requests: Stanley J. Szefler, MD, National Jewish Health, 1400 Jackson St., Rm. J304, Denver, CO 80206, Phone: (303) 398-1193, Fax: (303) 270 2189, Szeflers@njhealth.org .

Conclusion—LTE_4: FE_{NO} ratios predict a better response to MT than FP therapy in children with mild to moderate asthma.

Clinical Implications—In children with mild to moderate asthma, the LTE_4: FE_{NO} ratio is associated with a better response to montelukast compared to fluticasone therapy.

Capsule Summary—Data from 318 children with mild to moderate asthma enrolled in 2 NHLBI network studies (CLIC and PACT) were analyzed. Urinary LTE_4: FE_{NO} ratios predicted a better response to MT than FP therapy.

Keywords

asthma; biomarkers; fluticasone propionate; inhaled corticosteroids; leukotriene E4; montelukast

INTRODUCTION

Clinicians currently have two main alternatives for initiation of pharmacotherapy in children with mild to moderate persistent asthma. These choices are inhaled corticosteroids (ICS) or leukotriene receptor antagonists (LTRAs) especially montelukast (MT). Population studies and clinical trials have established that ICS therapy is more effective than LTRAs on almost all major health outcomes[1]. However, there is a subset of patients who appear to respond better to LTRAs with greater improvements in lung function2 and asthma control3 highlighting the need to detect predictive characteristics to identify this population.

In this context, the NHLBI CARE network CLIC2, 3 (Characterizing the Response to a Leukotriene Receptor Antagonist and an Inhaled Corticosteroid) and PACT4, 5 (Pediatric Asthma Controller Trial) studies were designed to identify predictors of ICS (i.e. fluticasone propionate (FP)) and MT responsiveness in school-aged children with mild to moderate disease. In these studies, characteristics of children with better responses to FP therapy were defined in a reproducible manner. Greater differential responses to FP over MT therapy were associated in both the CLIC and PACT studies with greater methacholine reactivity and higher levels of fractional exhaled nitric oxide (FE_{NO}).[2,5] Biological or physiological predictors of the subset of children who responded preferentially to MT therapy, however, could not be consistently identified.

A recent study of children with moderate to severe asthma predominantly receiving ICS therapy suggested that the ratio of 2 biomarkers, urinary leukotriene E4 (a marker of cysteinyl leukotriene (CysLT) production and elimination) and FE_{NO} (LTE_4: FE_{NO}) could help identify children who would respond to the addition of MT[6]. The authors of this study speculated that MT therapy might be most effective when the ratio of CysLT inflammation (as measured by urinary LTE_4) is high relative to eosinophilic inflammation (as measured by FE_{NO}). This observation highlighted the increased predictive value of utilizing a combination of biomarkers to predict therapeutic responses. The present analysis hypothesized that LTE_4: FE_{NO} ratios would be associated with a greater response to LTRA than to ICS therapy in children with mild to moderate persistent asthma.

METHODS

Study Protocols

Methods and primary outcome results for the CLIC2, 3 and PACT4, [5] studies have previously been extensively described. Appropriate institutional review board approval was obtained for each study before recruitment. Briefly, CLIC was a randomized crossover study of 127 children (6 to 17 years of age) with mild to moderate asthma. Blood for eosinophils, serum immunoglobulin E (IgE), eosinophilic cationic protein (ECP), FE_{NO} and urine for LTE_4 were

collected at randomization (one sample per child). Urinary LTE_4 was measured as described by Westcott et al.[7] Urine was treated with 50 milligrams (mg) of mouse antibody against the peptidoleukotrienes LTC_4, LTD_4, LTE_4, and N-acetyl LTE_4. The urine and antibody were allowed to incubate at room temperature for 4 to 5 hours. After filtering through a 10,000-micron cutoff filter, the LTE_4-antibody complex was separated by means of precipitation of the antibody with methanol. After evaporation of the methanol, an enzyme immunoassay was used for quantification. Urine LTE_4 levels were reported in picograms (pg) and standardized per mg of creatinine. FE_{NO} levels were measured with a rapid-response chemiluminescent analyzer (flow rate 50 milliliters/second; NIOX System, Aerocrine, Sweden) according to the guidelines of the American Thoracic Society.[8] Total serum IgE (Pharmacia CAP system) and eosinophil cationic protein (Pharmacia CAP system) levels were measured at a central site on serum from blood clotted at room temperature and then frozen and shipped.

Children were randomized either to active FP (100 micrograms (mcg) twice daily by diskus) with MT placebo or to active MT (5 or 10 mg) by mouth nightly (based on age recommendations) with FP placebo for an 8-week period at which time they were crossed over and treated for a second 8-week period. Asthma outcomes including forced expiratory volume in 1-second (FEV_1) and asthma control days (ACDs) were collected at randomization and every 4 weeks subsequently.

PACT was a parallel randomized study of 285 children (aged 6 to 14) with mild to moderate asthma. Asthma outcome data, FE_{NO} and urine for LTE_4 were collected (one sample per child) during a 2 to 4 week baseline characterization period and measured as described previously.[7,8] Children were randomized to 1 of 3 treatment arms (95 children in each arm); active FP (100 mcg twice daily by diskus), active MT (5 mg by mouth nightly), or active combination FP (100 mcg plus 50 mcg salmeterol each morning by diskus) and salmeterol (50 mcg nightly by diskus) with appropriate placebos. Children were treated for 12 months with asthma control indices collected every 6 weeks. For this analysis, only the FP and MT arms were evaluated.

Statistical Analysis

Demographic, biomarker or asthma control differences between CLIC and PACT studies were compared using chi-square (for categorical) or student-t tests for continuous variables. In order to compare PACT with CLIC and allow for a combined study analysis, analyses for the PACT study were restricted to data collected during the first 8 weeks post-randomization. The relationship between log-transformed LTE_4: FE_{NO}, and percent change in FEV_1 from baseline or change in average ACDs per week from the baseline period was evaluated. For comparison, levels of LTE_4, FE_{NO} and $1/FE_{NO}$ were also evaluated. FEV_1 measurements were performed 8 weeks after the start of each treatment phase in CLIC and 6 weeks after randomization in PACT, while mean ACDs were calculated from 4 to 8 weeks after the start of treatment in each study. An ACD was defined as a day without asthma symptoms, urgent care visits, hospitalizations, need for rescue albuterol (pre-treatment for exercise was permitted), or oral corticosteroids.

For CLIC data, the effect of each biomarker or biomarker ratio on the FEV_1 or ACD outcome was analyzed by treatment group using a mixed linear mixed-effects model to account for repeated measures as part of the study's cross-over design whereas the PACT data were analyzed using a linear regression model because it involved a parallel study design. For both studies, the estimates reported in the Results section represent regression slopes for the biomarkers, separately for MT and FP, with respect to the changes from baseline for the outcomes (FEV_1 and ACD). Next, the difference between the estimated regression slopes was calculated as a measure of the preferential response to MT (MT-FP). A meta-analytic weighted average was used to obtain a combined estimate from both studies, in which the weight for each study was set equal to the inverse of the squared standard error of the study estimate.

In addition to the predictive models, baseline characteristics of both CLIC and PACT children with high LTE_4: FE_{NO} (at or above the 75th percentile) were compared to other children using a Cochran-Mantel-Haenszel test for categorical variables and one way analysis of variance (ANOVA) for continuous variables. For these analyses, 273 CLIC and PACT participants in total were examined due to missing values of either LTE_4 or FE_{NO}. For all analyses, statistical significance was reported for p-values below 0.05.

RESULTS

In total, data from 318 children participating in CLIC (all children) or PACT (MT and FP arms only) were analyzed. At baseline, children in the PACT study were younger, had earlier age onset of asthma symptoms ($p<0.0001$) (Table I) and exhibited higher blood eosinophil levels ($p= 0.01$) and lower provocative concentrations of methacholine causing a 20% drop in FEV_1 (PC20s) ($p=0.04$) than children in the CLIC study (Table I). Although FEV_1 measurements were similar in CLIC and PACT, baseline ACDs were significantly lower in the PACT cohort ($p<0.0001$) (Table I). In addition, mothers of children in the PACT cohort were less likely to have smoked during pregnancy ($p=0.001$) (Table I). Biomarker levels were similar in both studies (Table I).

The distribution of biomarker levels and their logarithmically transformed values is presented in the online Table I. For CLIC, 8 of the 127 individuals were missing LTE_4 only, 14 were missing FE_{NO} only, and 1 was missing both LTE_4 and FE_{NO}. For PACT, 13 of the 191 individuals were missing LTE_4 only, 9 were missing FE_{NO} only, and 0 were missing both LTE_4 and FE_{NO}. Median levels for FE_{NO} measured 26.4 parts per billion (ppb) consistent with asthma. For LTE_4, median levels were 100 pg/mg, for $1/FE_{NO}$, 0.04 ppb, and for the LTE_4: FE_{NO} ratio, 3.9 pg/mg/ppb.

Relationship between Biomarker Predictors and Treatment Response

FEV_1 Response—Table II summarizes the association between each of the biomarker predictors and the FEV_1 percent change from baseline. In the CLIC study, LTE_4: FE_{NO} ratios were associated with a significant MT response from baseline (1.7% increase per doubling of the ratio, $p=0.02$) and a greater MT than FP response (2.1% increase, $p=0.001$). FE_{NO} levels were significantly associated with a greater FEV_1 response to FP than MT (1.8% increase for each doubling of biomarker at baseline, $p=0.004$). In the PACT study, LTE_4: FE_{NO} ratios were inversely associated with FP response (0.6% decrease, $p=0.001$) and positively associated with a greater MT than FP response (0.6% increase, $p= 0.03$). In addition, FP response was associated with LTE_4 (2.8% increase, $p=0.03$), or FE_{NO} (0.4% increase, $p= 0.02$) levels and inversely associated with $1/FE_{NO}$ (7.6% decrease, $p= 0.02$). In the combined study meta-analysis, LTE_4: FE_{NO} ratios were significantly associated with a greater MT than FP response (0.8% increase, $p=0.0005$) and inversely associated with FP response (0.6% decrease, $p=0.0008$). LTE_4 levels were associated with a significant MT (2.4% increase, $p=0.01$) or FP (2.4% increase $p=0.01$) response but not to a differential response ($p=0.68$). FE_{NO} levels were associated with FP response (0.4% increase, $p=0.01$) and to a greater FP than MT response (0.6% increase, $p=0.01$) while 1/ FE_{NO} levels were inversely associated with FP response (7.1% decrease, $p=0.02$).

ACD Response—Table III summarizes the association between each of the biomarker predictors and changes in average ACDs per week from baseline. In the CLIC study, LTE_4: FE_{NO} ratios were associated with a greater MT than FP response (0.3 ACD increase per doubling of the ratio, $p=0.009$) and FE_{NO} levels were associated with a greater FP than MT response (0.3 ACD increase, $p= 0.01$) while 1/ FE_{NO} levels were inversely associated with FP

response (4.9 ACD decrease, p= 0.02). There were no significant associations observed in the PACT study for any of the predictors.

In the combined study meta-analysis, LTE_4: FE_{NO} ratios were significantly associated with a greater response to MT than FP therapy (0.3 ACD increase, p= 0.008). In addition, FE_{NO} levels were associated with a significant FP response (0.4 ACD increase, p= 0.009) and to a greater FP than MT response (0.3 ACD increase, p=0.009) while 1/ FE_{NO} levels were inversely associated with FP response (5.2 ACD decrease, p=0.04).

Demographic Characteristics of Children with High LTE_4: FE_{NO} Ratios

Table IV relates the demographic characteristics measured at baseline of children with high (>/= 75th percentile) LTE_4: FE_{NO} versus lower ratios. Children with high ratios were more likely to be younger (p<0.0001) and female (p=0.03). Children with high LTE_4: FE_{NO} ratios were less likely to be atopic (i.e. exhibit 1 or more positive aeroallergen skin tests)(p<0.0001), and had fewer positive aeroallergen skin tests (p<0.0001), lower serum IgE levels (p=0.0002), less percent blood eosinophils (p<0.0001), lower serum eosinophilic cationic protein (p=0.004) and higher methacholine PC_{20} (p=0.02) as compared to those with lower LTE_4: FE_{NO} ratios. The LTE_4: FE_{NO} ratio remained as a significant predictor of the differential MT response after control for atopy, serum IgE levels and percent blood eosinophils (Online Table II).

DISCUSSION

The National Asthma Education and Prevention Program guidelines recommend ICS as the preferred controller therapy for persistent asthma in adults and children[1]. Despite this recommendation, the LTRA montelukast is the most frequently prescribed asthma controller monotherapy in the United States[9] although consistent findings in both population studies and clinical trials report greater efficacy and effectiveness of ICS than LTRA therapy. This prescribing trend probably reflects parent and provider's preference for an oral non-corticosteroid medication instead of an ICS inhaler due to the convenience of an oral medication and concern for potential growth effects associated with ICS.[10] Identification of variables predictive of greater responsiveness to LTRA than ICS therapy could help providers recommend ICS therapy in the majority of patients consistent with national guidelines while selecting only the subset of children who might respond more effectively to LTRA therapy.

The aim of this secondary analysis was to test the hypothesis that the LTE_4: FE_{NO} ratio could predict preferential MT over FP responsiveness. As such, the study assessed consistency with both FEV_1 and ACD health outcomes as well as reproducibility across different study groups by first analyzing data from the crossover CLIC study and then checking for reproducibility of these findings using data from the PACT parallel study. In this context, a statistically significant association between LTE_4: FE_{NO} ratios and preferential MT response was observed for both FEV_1 and ACD outcome measures with CLIC data. The association with a preferential FEV_1 response to MT was than reproduced using data from the PACT study. Although significant associations with ACDs were not observed in PACT, this may have been due to inadequate power as PACT data produced relatively large standard errors for this health outcome. In contrast to these findings, significant associations were not observed with individual ratio components (i.e. LTE_4 and $1/FE_{NO}$) in CLIC or PACT indicating that the ratio of LTE_4 and FE_{NO} had greater predictive value than either component alone. Significant associations with preferential FEV_1 and ACD responsiveness were also observed with the LTE_4: FE_{NO} ratio when data from CLIC and PACT studies were combined using a meta-analytic approach. Although further work is needed to confirm its clinical value, these results support the proof of concept for measuring the LTE_4: FE_{NO} ratio as a predictor of preferential MT responsiveness in children with mild to moderate asthma.

Previous studies have assessed the utility of individual biomarkers such as urinary LTE_4 and FE_{NO} to delineate heterogeneous patient profiles of inflammation that may respond differently to medications. These studies and the data presented here suggest that higher LTE_4 levels might be related to either MT or FP therapy responsiveness (although these patterns were not consistently observed), while FE_{NO} levels were associated with FP response only. In contrast, LTE_4: FE_{NO} ratios were associated with a better response to MT than to FP therapy. As such, we speculate that urinary LTE_4 is a more general indicator of the inflammatory response to multiple triggers while allergic-type triggers specifically increase FE_{NO} levels. In this context, high LTE_4: FE_{NO} ratios were related to a lower degree of IgE sensitization, lower levels of eosinophilic markers and less responsiveness to ICS treatment while retaining sensitivity to LTRA therapy.

This phenotype might be related to non-allergic asthma triggers such as tobacco smoke exposure that may increase urinary LTE_4 levels[11] and decrease FE_{NO} concentrations.[12] In this context, previous reports have suggested increased MT responsiveness in smokers[13] or in children exposed to environmental tobacco smoke.[6], Although associations were not observed with questionnaire data from CLIC and PACT, urinary cotinine measurements were not available to allow for a definitive assessment of the relationship between smoking exposure, higher ratios of LTE_4: FE_{NO} and preferential MT responsiveness.

Additionally, FE_{NO} may be considered to be a marker of allergen-driven, local bronchial inflammation (readily targeted by an ICS), whereas urinary LTE_4 may originate in the systemic circulation as well as in the airways. Systemic inflammation can be elicited by rhinovirus infections through the release of IP-10 (and rhinovirus-induced effects are at least partially resistant to treatment with ICS) [14]. Thus, urinary LTE_4 may signal a viral-induced systemic component that is targeted by MT[15] but not FP. Because viral specimens were not collected in the CLIC and PACT studies, the relationship between viral exposure, higher ratios of LTE_4: FE_{NO} and preferential MT responsiveness could not be assessed.

In addition to marking a phenotype with less IgE sensitization and lower levels of eosinophilic markers, high LTE_4: FE_{NO} ratios were more likely to occur in girls. This observation is consistent with some reports of increased likelihood of response to MT in females[6,15] and suggests that differential hormonal patterns might be related to inflammatory heterogeneity in asthma. In addition, younger age was related to high LTE_4: FE_{NO} ratios probably reflecting the fact that urinary LTE_4 levels decrease[16] while FE_{NO} levels increase[17] with age due to the larger bronchial mucosa area available for FE_{NO} diffusion in older children. In the CLIC study, [2] younger children were more likely to respond to MT while older children participating in the PACT study[5] tended to respond better to FP than MT therapy. These age-related trends support the need for future studies among pre school children, infants and toddlers who often manifest with frequent viral-induced exacerbations that are poorly responsive to ICS therapy.[18] In this context, Bisgaard et al. reported that asthma exacerbations[19] and symptoms of cough and wheeze after bronchiolitis in infants [20] were reduced significantly with MT therapy. As such, determining the ratio of urinary LTE_4 to FE_{NO} (or perhaps a related biomarker that can be measured in an effort-independent manner) could help identify a relatively large subset of younger patients who would derive greater benefit from LTRA therapy.

CLIC and PACT investigators had previously reported on potential demographic predictors of preferential MT response but results were not consistent across asthma outcomes or across studies. For example, in the CLIC study, younger age and female gender were significant predictors for FEV_1 response to MT and higher PC20s were significantly associated with a preferential FEV_1 response to MT but these results were not reproducible in PACT. No significant demographic predictors of ACD response to MT treatment or preferential MT response were observed in either study. As such, although the present study suggests that these

previously reported demographic associations might reflect higher LTE_4: FE_{NO} ratios, such demographic characteristics alone cannot be used as consistent and reproducible predictors of preferential MT response.

Enzyme immunoassays have been shown to be a sensitive method for measurement of LTE_4. The immunoassay utilized in this report has also been used in a number of other pediatric asthma reports [6,21–24]. It employs enrichment methods to purify the urine and increase assay specificity. Despite these improvements, the LTE_4 immunoassay used in this study is labor intensive and sometimes produces higher readings suggesting poorer specificity than those obtained with solid phase extraction and reverse phase high performance liquid chromatography.[7] Although multiple repeated biomarker measurements for each subject were not performed in CLIC and PACT, Rabinovitch et al. [6,23] have previously reported that daily changes in LTE_4 measurements were associated with individual changes in FEV_1 or rescue medication use. Unpublished observations by these authors using this repeated measures data found moderate but not excellent intraclass correlations for LTE_4 measured by immunoassay. These observations indicate the need for a more precise LTE_4 assay and/or repeated LTE_4 measurements to maximize precision and minimize measurement error which may produce estimates biased towards the null. As the LTE_4: FE_{NO} ratio was consistently associated with differential montelukast response despite the immunoassay limitations, these findings should be seen as an important proof-of concept supporting the need for further assay refinement and standardized collection methods. Similarly, when the use of exhaled nitric oxide levels in asthma was first studied, collection methods were expensive and unstandardized. As the clinical relevance of exhaled nitric oxide levels became clearer, the technology advanced fairly quickly and became more user-friendly. Fortunately, new high-throughput and less labor intensive methods for measuring LTE_4 in urine based on automated sample enrichment and liquid chromatography/tandem mass spectrometry have now been developed which have lower coefficients of variation and almost 100% recovery using spiked samples indicating high sensitivity and precision.[16, 25] One would expect even stronger predictive ability for the LTE_4: FE_{NO} ratio measured with these more precise and efficient assays. Further studies utilizing these more refined assays are planned to identify cut-off points for guiding therapeutic decisions.

Because this study used a continuous instead of a categorical approach as a proof of concept, it did not select a cut-off point to determine the number of doublings of the ratio required to predict a clinically significant differential response nor is such a value for differential responses well defined in the literature. The report by Zeiger et al. [3], using the CLIC crossover data, proposed 1 extra ACD per week as defining a clinically significant differential response. Using this definition, 29% of children in the CLIC study achieved a 1 ACD or greater differential FP response, 12% achieved a better MT response and 59% of children responded similarly (within 1 ACD/week) to either medication. Rather than basing treatment decisions solely on the average response, measuring the biomarker ratio allows for identification of children in the tails of the population distribution (e.g., below the 10^{th} percentile and above the 90^{th} percentile for MT-FP responders). As such, the FE_{NO} variable alone could be utilized to identify the 29% of children with a clinically significant differential ACD response to FP therapy (FP-MT) and the LTE_4: FE_{NO} ratio to identify those 12% with a better MT response (MT-FP). Given the 0.3 ACD increase per week per doubling dose estimate for both CLIC alone and for the combined study population using the meta-analytic approach, this translates into 3.3 doubling doses or greater to achieve at least a 1ACD per week difference in the MT-FP response.

In summary, ratios of LTE_4: FE_{NO} were associated with a greater FEV_1 and ACD response to MT than FP therapy in analysis of 2 randomized studies of 318 schoolchildren with mild to moderate asthma. Children with LTE_4: FE_{NO} ratios at or above the 75^{th} percentile were younger, more likely to be female, and exhibited lower levels of atopic markers and bronchial

hyper reactivity. Although clinical guidelines support use of ICS therapy as first line controller therapy in mild to moderate persistent childhood asthma, measurement of LTE_4: FE_{NO} ratios might be useful in identifying individual children who achieve a greater improvement in FEV_1 and ACDs with an LTRA compared to an ICS.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

ANOVA	Analysis of variance
ACD	asthma control days
CLIC	Characterizing the Response to a Leukotriene Receptor Antagonist and an Inhaled Corticosteroid
CysLT	cysteinyl leukotriene
ETS	environmental tobacco smoke
FP	fluticasone propionate
LTE_4	leukotriene E_4
FE_{NO}	fractional exhaled nitric oxide
FEV_1	forced expiratory volume in 1-second
LTRA	leukotriene receptor antagonist
mcg	micrograms
mg	milligram
MT	montelukast
ppb	Parts per billion
PACT	Pediatric Asthma Controller Trial
pg	pictogram
PC_{20}	provocative concentration of methacholine causing a 20 % drop in FEV_1

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Table I

Baseline Characteristics For CLIC and PACT Participants

Baseline Characteristic	CLIC	PACT (only includes those participants randomized to MT or FP)	P-value* comparing CLIC and PACT	CLIC and PACT Combined
Randomized participants, n	127	191	---	318
Age, years	11.8 ± 3.35	9.7 ± 2.21	<0.0001	10.5 ± 2.91
Male gender, n (%)	75 (59.1%)	114 (59.7%)	0.91	189 (59.4%)
Caucasian, n (%)	95 (74.8%)	149 (78.0%)	0.51	244 (76.7%)
Hispanic or Latino, n (%)	31 (24.4%)	36 (18.9%)	0.23	67 (21.1%)
BMI (kg/m^2)	20.9 ± 4.95	19.9 ± 4.93	0.06	20.3 ± 4.96
Age at onset asthma symptoms, years	4.8 ± 3.70	3.2 ± 2.78	<0.0001	3.8 ± 3.27
Mother smoked while pregnant, n (%)	25 (19.7%)	14 (7.3%)	0.001	39 (12.3%)
Household member smokes indoors, n (%)	22 (17.3%)	23 (12.0%)	0.19	45 (14.2%)
Parent asthma diagnosis, n (%)	57 (49.6%)	96 (50.3%)	0.10	153 (50.0%)
Eczema, n (%)	40 (31.5%)	79 (41.4%)	0.07	119 (37.4%)
Positive aeroallergen skin tests (of 8)	2.9 ± 2.24	2.5 ± 2.05	0.09	2.6 ± 2.13
Serum IgE (k U/L) (Log2)	7.1 ± 2.08	7.2 ± 2.15	0.88	7.1 ± 2.11
Blood eosinophils (%)	4.9 ± 3.41	6.0 ± 3.89	0.01	5.6 ± 3.73
Serum eosinophilic cationic protein (mcg/l) (Log2)	4.0 ± 1.18	4.0 ± 1.42	0.99	4.0 ± 1.33
Urinary LTE_4 (pg/mg creatinine) (Log2)	6.7 ± 0.67	6.6 ± 0.74	0.39	6.6 ± 0.71
FE_{NO} (ppb) (Log2)	4.7 ± 1.39	4.7 ± 1.36	0.87	4.7 ± 1.37
Pre-bronchodilator FEV_1 % predicted	95.8 ± 13.26	97.8 ± 12.86	0.19	97.0 ± 13.03
Pre-bronchodilator FEV_1/FVC (%)	80.1 ± 7.84	80.1 ± 7.86	0.97	80.1 ± 7.84
Average asthma-control days per week	2.2 ± 2.33	0.3 ± 0.23	<0.0001	1.1 ± 1.76
Average night awakenings per week	0.3 ± 0.55	0.0 ± 0.08	<0.0001	0.1 ± 0.37
Methacholine PC_{20} (mg/ml)	2.7 ± 3.80	2.0 ± 2.57	0.04	2.3 ± 3.13

*P-values calculated using chi-square test (for categorical variables) or student's t-test (for continuous variables).

Table II

Relationship between LTE_4: FE_{NO} Levels and Percent Change in FEV_1 *

	CLIC (N=127)			PACT (N=191)			CLIC + PACT (meta-analytic results) (N=318)		
	MT	**FP**	**Difference (MT - FP)**	**MT**	**FP**	**Difference (MT - FP)**	**MT**	**FP**	**Difference (MT - FP)**
Log2 (LTE_4: FE_{NO})									
Estimate	1.7	−0.4	+2.1	0.0	−0.6	+0.6	0.1	−0.6	+0.8
(Std. Error)	(0.71)	(0.70)	(0.63)	(0.18)	(0.18)	(0.25)	(0.17)	(0.17)	(0.23)
P-value**	0.02	0.58	0.001	0.92	0.001	0.03	0.55	0.0008	0.0005
Log2 LTE_4									
Estimate	1.9	2.3	−0.5	2.5	2.8	−0.3	2.4	2.4	−0.4
(Std. Error)	(1.35)	(1.35)	(1.27)	(1.29)	(1.29)	(1.8)	(0.93)	(0.93)	(1.04)
P-value**	0.17	0.09	0.72	0.06	0.03	0.87	0.01	0.01	0.68
Log2 FE_{NO}									
Estimate	−0.9	0.9	−1.8	0.0	0.4	−0.4	−0.1	0.4	−0.6
(Std. Error)	(0.72)	(0.72)	(0.61)	(0.18)	(0.18)	(0.26)	(0.17)	(0.17)	(0.24)
P-value**	0.22	0.21	0.004	0.92	0.02	0.12	0.76	0.01	0.01
1 / (Log2 FE_{NO})									
Estimate	3.1	−3.4	+6.5	−0.1	−7.6	+7.5	0.2	−7.1	+7.3
(Std. Error)	(9.07)	(9.06)	(7.85)	(2.86)	(3.18)	(4.28)	(2.73)	(3.00)	(3.76)
P-value**	0.74	0.71	0.41	0.98	0.02	0.08	0.94	0.02	0.05

* CLIC = 2nd 4 weeks of data in each treatment phase; PACT = first 6 weeks of data after randomization to a treatment

** P-values computed using a regression model to see if estimate (slope) is significantly different from zero. Comparison of CLIC data and CLIC+PACT data uses a mixed linear model to account for repeated measures among CLIC subjects.

Table III

Relationship between LTE_4: FE_{NO} Levels and Change in Asthma Control Days Per Week *

	CLIC (N=127)			PACT (N=191)			CLIC + PACT (meta-analytic results) (N=318)		
	MT	**FP**	**Difference (MT - FP)**	**MT**	**FP**	**Difference (MT - FP)**	**MT**	**FP**	**Difference (MT - FP)**
Log2 (LTE_4: FE_{NO})									
Estimate	0.2	−0.1	+0.3	0.9	−0.2	+1.2	0.2	−0.1	+0.3
(Std. Error)	(0.18)	(0.18)	(0.12)	(0.70)	(0.69)	(0.98)	(0.17)	(0.17)	(0.12)
P-value**	0.60	0.19	0.009	0.18	0.73	0.23	0.16	0.54	0.008
Log2 LTE_4									
Estimate	0.6	0.7	−0.1	0	−0.4	+0.3	0.2	0.1	0
(Std. Error)	(0.35)	(0.35)	(0.27)	(0.35)	(0.34)	(0.49)	(0.21)	(0.24)	(0.24)
P-value**	0.09	0.05	0.73	0.89	0.27	0.50	0.30	0.61	0.98
Log2 FE_{NO}									
Estimate	0	0.4	−0.3	−0.2	0.9	−1.0	0	0.4	−0.3
(Std. Error)	(0.17)	(0.17)	(0.12)	(0.68)	(0.67)	(0.95)	(0.16)	(0.16)	(0.12)
P-value**	0.84	0.05	0.01	0.82	0.19	0.28	0.99	0.009	0.009
1 / (Log2 FE_{NO})									
Estimate	−3.0	−4.9	+2.0	2.8	−15.0	+17.9	−2.8	−5.2	+2.1
(Std. Error)	(2.15)	(2.15)	(1.52)	(10.60)	(12.32)	(16.25)	(2.11)	(2.12)	(1.51)
P-value**	0.17	0.02	0.20	−0.79	0.22	0.27	0.19	0.01	0.16

*CLIC = 2nd 4 weeks of data in each treatment phase; PACT = weeks 4–8 of data after randomization to a treatment

**P-values computed using a regression model to see if estimate (slope) is significantly different from zero. Comparison of CLIC data and CLIC+PACT data uses a mixed linear model to account for repeated measures among CLIC subjects.

Table IV

Baseline Characteristics of Children with High (at or above the 75th percentile) LTE_4: FE_{NO} Levels

Baseline Characteristic	Log2 (LTE_4: FE_{NO}) (pg/mg/(ppb))		P-value*
	Lower ratio < 2.89	Higher ratio ≥ 2.89	
Participants, n	205	68	
Age, years	10.9 ± 2.89	9.2 ± 2.44	<0.0001
Male gender, n (%)	131 (63.9%)	33 (48.5%)	0.03
Caucasian, n (%)	161 (78.5%)	48 (70.6%)	0.77
Hispanic or Latino, n (%)	43 (21.0%)	13 (19.1%)	0.74
BMI (kg/m^2)	20.3 ± 4.68	20.7 ± 5.68	0.55
Age at onset asthma symptoms, years	4.2 ± 3.43	3.4 ± 3.15	0.10
Mother smoked while pregnant,n (%)	21 (10.2%)	6 (8.8%)	0.89
Household member smokes indoors, n (%)	26 (12.7%)	9 (13.2%)	0.91
Parent asthma diagnosis, n (%)	103 (51.8%)	28 (45.2%)	0.42
Eczema, n (%)	78 (38.1%)	23 (33.8%)	0.53
Atopy, n (%)	180 (87.8%)	39 (57.3%)	<0.0001
Positive aeroallergen skin tests (of 8)	3.0 ± 1.94	1.8 ± 2.21	<0.0001
Log2 Serum IgE (k U/L)	229.0 ± 365.89	58.4 ± 142.34	0.0002
Blood eosinophils (%)	6.3 ± 3.77	3.2 ± 2.20	<0.0001
Serum eosinophilic cationic protein (mcg/l)	26.5 ± 32.07	15.0 ± 11.78	0.004
Pre-bronchodilator FEV_1 % predicted	97.1 ± 12.27	99.4 ± 14.96	0.20
Pre-bronchodilator FEV_1/FVC (%)	79.9 ± 7.83	81.6 ± 7.58	0.13
Average asthma-control days per week	1.0 ± 1.74	1.1 ± 1.59	0.74
Average night awakenings per week	0.1 ± 0.35	0.1 ± 0.40	0.83
Methacholine PC_{20} (mg/ml)	2.0 ± 2.76	2.9 ± 2.93	0.02

*P-values calculated using Cochran-Mantel-Haenszel Tests for categorical variables and 1-way ANOVA for continuous variables.