

Exhaled breath condensate eicosanoid levels associate with asthma and its severity

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Background: The relationship between anti-inflammatory lipoxins and proinflammatory leukotrienes might be important in the pathobiology and severity of asthma.

Objective: We sought to investigate whether exhaled breath condensate (EBC) lipoxin and leukotriene measurements can noninvasively characterize the asthmatic diathesis and its severity.

Methods: We measured lipoxin A₄ (LXA₄) and leukotriene B₄ (LTB₄) levels in EBC collected from patients with asthma of different severities and from healthy control subjects.

Results: EBC LXA₄ and LTB₄ levels are increased in asthmatic patients compared with those seen in healthy control subjects (LXA₄: 31.40 vs 2.41 pg/mL EBC, respectively [$P < .001$]; LTB₄: 45.62 vs 3.82 pg/mL EBC, respectively [$P < .001$]). Although levels of both eicosanoids are increased in asthmatic patients, the LXA₄/LTB₄ ratio decreases with increasing asthma severity. It is 41% lower in patients with severe versus moderate asthma

(0.52 vs 0.88, $P = .034$). EBC LXA₄ levels correlate with the degree of airflow obstruction measured by using FEV₁ ($r = 0.28$, $P = .018$). An LXA₄ cutoff value of 7 pg/mL EBC provides 90% sensitivity and 92% specificity for the diagnosis of asthma (area under the curve, 0.96; $P < .001$). An LTB₄ cutoff value of 11 pg/mL EBC provides 100% sensitivity and 100% specificity for the diagnosis of asthma (area under the curve, 1; $P < .001$). **Conclusions:** Proresolving and proinflammatory eicosanoids are generated in the airways of all asthmatic patients. The proportion of proresolving compounds decreases with asthma severity. These findings support the role for EBC eicosanoid measurements in the noninvasive diagnosis of asthma and suggest that proresolving eicosanoid pathways are dysregulated in patients with severe asthma. (J Allergy Clin Immunol 2013;■■■■:■■■-■■■.)

Key words: Asthma, biomarkers, breath tests, eicosanoids, leukotriene B₄, lipoxin A₄

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Airway inflammation in asthmatic patients is characterized by upregulation of multiple inflammatory mediators and cytokines. Among the most potent proinflammatory mediators are arachidonic acid-derived leukotrienes.¹ In addition to being potent chemoattractants and activators of inflammatory cells, such as neutrophils and eosinophils, they are also among the most potent bronchoconstrictors identified.²

We now understand that accompanying the activation of inflammatory cascades is the generation of proresolving mediators, which restore tissue homeostasis through antagonism of inflammation and promotion of immune defense mechanisms.³ Among these moieties are lipoxins, lipoxygenase interaction products of arachidonic acid metabolism. Of particular relevance to asthma, they inhibit eosinophils, neutrophil responses, and cytokine signaling.⁴ They also antagonize the effects of leukotrienes; they inhibit chemotactic responses of human neutrophils and eosinophils stimulated by leukotriene B₄ (LTB₄), act as endogenous antagonists at the cysteinyl leukotriene (CysLT) 1 receptor, and modulate leukotriene C₄-induced airway obstruction.⁵⁻⁹

Lipoxins are generated in the inflamed asthmatic lung; however, their inadequate production, function, or both is associated with the more severe phenotype of asthma.¹⁰⁻¹⁷ Patients with severe asthma have reduced lipoxin levels in whole blood, bronchoalveolar lavage (BAL) fluid, and sputum.^{10,11,16} We have also found that lipoxin deficiency is directly proportional to loss of lung function in asthmatic patients.¹⁰ Limitations in the procurement of lung-localized specimens have been significant barriers in advancing investigations into the role of dysregulated eicosanoid metabolism in asthmatic patients.

Exhaled breath condensate (EBC) collection offers a noninvasive method to sample the respiratory epithelial lining fluid.¹⁸ Biomarkers measured in EBC can provide information about specific biological processes associated with pulmonary diseases,

Abbreviations used

BAL: Bronchoalveolar lavage
 CysLT: Cysteinyl leukotriene
 EBC: Exhaled breath condensate
 FENO: Fraction of exhaled nitric oxide
 ICS: Inhaled corticosteroid
 LTB₄: Leukotriene B₄
 LXA₄: Lipoxin A₄

their severity, and response to therapy.¹⁹ Lipid-based inflammatory mediators, including lipoxins and leukotrienes, have been successfully isolated from EBC.^{19–21} Therefore we hypothesized that we could use noninvasively collected EBC to assess the relationship between the lipoxin and leukotriene pathways in asthmatic diathesis and its severity.

Herein we present data that suggest that both inflammatory and proresolving eicosanoid pathways are upregulated in the asthmatic airway and that relative underproduction of lipoxins occurs with increasing asthma severity. In addition to providing insight into the pathobiology of asthma, these data also suggest that noninvasive EBC measurements can be used as sensitive and specific biomarkers to distinguish asthma from health.

METHODS**Participants**

All participants provided written informed consent that was approved by the institutional review board. We recruited adults with mild intermittent, moderate, and severe asthma to participate in a phenotypic characterization study as part of the National Heart, Lung, and Blood Institute's Severe Asthma Research Program. Severe asthma was defined per the criteria developed by the American Thoracic Society Workshop on Refractory Asthma.²² Patients with moderate asthma were required to be taking low- to medium-dose inhaled corticosteroids (ICSs), as defined by the National Asthma Education and Prevention Program guidelines (<880 µg of fluticasone or equivalent).²³ Patients with mild asthma had intermittent symptoms controlled without the use of anti-inflammatory medications. Patients with a history of other respiratory diseases, including allergic bronchopulmonary aspergillosis, were excluded from the study. Adults without a prior history of asthma, allergies, or chronic respiratory symptoms were recruited as healthy control subjects. All participants were required to have a 5 pack-year or less smoking history.

Baseline spirometry was performed in asthmatic patients after bronchodilator withhold if their asthma symptoms permitted (4 hours for short-acting β-agonists and 12 hours for long-acting β-agonists). Subjects were not asked to withhold montelukast or ICSs unless the latter were prescribed in combination with their long-acting β-agonist.

Asthmatic patients had to demonstrate either bronchodilator reversibility of at least 12% in FEV₁, a methacholine PC₂₀ value of 8 mg/mL or less, or hyperresponsiveness to 3% saline challenge with a PD₂₀ value of 16 mL or less. Additional phenotypic characterization for participants with asthma included assessment for aspirin sensitivity and measurement of serum IgE levels, peripheral blood eosinophil counts, and fraction of exhaled nitric oxide (FENO) levels.

EBC collection

EBC was collected once from each enrolled study participant by using recommended methods with an RTube (Respiratory Research, Austin, Tex).¹⁸ Participants were required to withhold food and carbonated beverages for 1 hour before EBC collection. The metal sleeve surrounding the collection chamber was chilled to –20°C for at least 1 hour before EBC collection. Samples were collected by using tidal breathing through the mouth for 10 minutes without a nose clip. Eicosanoid loss was minimized by rapidly placing samples in aliquots and freezing them at –80°C under a layer of inert gas immediately subsequent to

the 10-minute collection. EBC samples from participants with asthma were stored for approximately 2 years before analyses. EBCs from healthy control subjects were stored for approximately 6 months before analyses.

Extraction and measurement of lipid mediators

Eicosanoids were extracted from EBC by using C₁₈ Sep-Pak cartridges (Waters, Milford, Mass) and concentrated, as previously described.¹⁰ Lipoxin A₄ (LXA₄), LTB₄, and CysLT concentrations were determined by using enzyme-based immunoassays (Neogen, Lexington, Ky, and Cayman Chemical, Ann Arbor, Mich). Manufacturer-provided lower limits of detection for the assays were 20 pg/mL for LXA₄, 13 pg/mL for LTB₄, and 34 pg/mL for the CysLTs; however, we were able to detect levels as low as 2 pg of LXA₄, 2 pg of LTB₄, and 5 pg of CysLT per milliliter of EBC because the extracted mediators were concentrated up to 10-fold before measurement. Mediator values were recalculated to reflect the amount per milliliter of EBC before concentration and normalized to the denominator "per milliliter of EBC" independent of the volume of fluid collected from a participant. If the recalculated mediator level was greater than or less than the adjusted limits of detection of an assay for a concentrated sample, we assigned them the following values. Those less than the lower limit of detection were assigned a value of half of the adjusted lower limit for the concentrated sample. Those greater than the upper limit of detection were assigned a value twice as much as the adjusted upper limit for the concentrated sample.

Statistical analyses

All samples were deidentified before measurements. We transformed all mediator values to their logarithm at base 10 to normalize the distributions before analysis. Normality was determined by examining the histogram of the distribution of the outcomes and the *P* value obtained by using the Shapiro-Wilk test. We performed analyses of covariance for each transformed outcome. Each was adjusted separately for age, sex, and race. Categorical data were analyzed by using the χ² and Fisher exact tests. Student *t* tests were used for normally distributed continuous data, whereas nonparametric data were analyzed by using the Wilcoxon rank sum, Kruskal-Wallis, and Spearman correlation tests. Cutoffs for optimal sensitivity and specificity were determined by examination of receiver-operating curves. We used GraphPad Prism 6.00 (GraphPad Software, La Jolla, Calif) and SAS 9.2 (SAS Institute, Cary, NC) software to examine and analyze the data. The significance level used in these tests was set at 95%. For continuous data analyzed by using parametric or nonparametric methods, we report the arithmetic means ± SDs, with minimum and maximum values for the observations shown in parentheses. For skewed data that were log transformed before analyses, we report geometric means with the 95% CIs in parentheses. For count data, we report the number in each category, with the percentage of total in parentheses.

RESULTS**Participants' characteristics**

Tables I and II describe the demographic and phenotypic characteristics of the participants. Patients with severe asthma were the oldest among the 4 groups. Groups were similar with respect to the distribution of sex, race, and ethnicity. As expected, patients with severe asthma received much higher doses of ICSs and had lower lung function. There were no other significant differences between the patients with moderate and those with severe asthma, similar to what has been seen in the entire Severe Asthma Research Program cohort's phenotypic characterization.²⁴ None of the asthmatic patients were receiving oral corticosteroids, anti-IgE therapy, or the 5-lipoxygenase inhibitor zileuton.

EBC LXA₄ levels

EBC LXA₄ levels were more than 10-fold higher in the asthmatic patients compared with those seen in healthy control

TABLE I. Participants' demographics

	Healthy control subjects	Patients with mild asthma	Patients with moderate asthma	Patients with severe asthma	P value for difference between groups
No.	12	10	23	36	
Age (y)*	27 ± 4 (23-36)	33 ± 12 (18-57)	33 ± 10 (18-53)	42 ± 11 (21-62)	<.001
Sex, female/male (%)	7 (58)/5 (42)	6 (60)/4 (40)	16 (70)/7 (30)	22 (61)/14 (39)	.886
Race, white/African American/other (%)	7 (58)/1 (9)/4 (33)	9 (90)/0 (0)/1 (10)	16 (69)/5 (22)/2 (9)	19 (52)/11 (31)/6 (17)	.154
Ethnicity, Hispanic/non-Hispanic/unknown (%)	0 (0)/12 (100)/0 (0)	1 (10)/8 (80)/1 (10)	1 (4)/21 (92)/1 (4)	7 (19)/29 (81)/0 (0)	.230
Sensitivity to aspirin, yes/no/unknown (%)	NA	0 (0)/10 (100)/0 (0)	2 (9)/15 (65)/6 (26)	3 (8)/17 (47)/16 (45)	.705
Daily dose of inhaled fluticasone equivalent (μg)*	NA	NA	392 ± 142 (176-500)	1004 ± 156 (880-1880)	<.001
Montelukast use, yes/no (%)	NA	NA	9 (39)/14 (61)	17 (47)/19 (53)	.559

Data were analyzed by means of ANOVA, the Student *t* test, and the Fisher exact test.

NA, Not applicable.

*Data are presented as arithmetic means ± SDs (minimum-maximum).

TABLE II. Asthma phenotypic characteristics

	Healthy control subjects	Patients with mild asthma	Patients with moderate asthma	Patients with severe asthma
No.	12	10	23	36
FEV ₁ (L)*	ND	2.93 ± 0.68	3.16 ± 0.79	2.38 ± 0.79‡
FEV ₁ (% predicted)*	ND	85 ± 13	93 ± 16	71 ± 18‡
FVC (L)*§	ND	3.48 ± 0.91	3.91 ± 0.97	3.32 ± 0.90‡
FVC (% predicted)*§	ND	84 ± 18	96 ± 14	81 ± 14‡
FEV ₁ /FVC ratio*§	ND	84 ± 9	81 ± 6	71 ± 10‡
Two-puff bronchodilator reversibility (%)*	ND	ND	8 ± 9	13 ± 12
Methacholine PC ₂₀ (mg/mL)†	ND	ND	1.89 (1.00-3.6)	2.40 (1.45-4.00)
Exhaled nitric oxide (ppb)*	ND	ND	22.74 ± 14.27	28.53 ± 23.79
Serum IgE level (IU/mL)*¶	ND	ND	226 ± 344	344 ± 434
Peripheral blood eosinophils (%)*	ND	ND	4 ± 3 (0-10)	4 ± 2 (0-11)

FVC, Forced vital capacity; ND, not done.

*Data are presented as arithmetic means ± SDs.

†PC₂₀ is defined as the percentage concentration required to cause a 20% decrease in FEV₁. Data are presented as geometric means (95% CIs), with 21 patients with nonsevere asthma and 23 patients with severe asthma for methacholine PC₂₀ results. Data were analyzed after logarithmic conversion.

‡*P* < .001 compared with patients with moderate asthma for the difference in means calculated by using the unpaired Student *t* test.

§N = 9 for patients with mild asthma.

||N = 22 for patients with moderate and 31 for patients with severe asthma.

¶N = 35 for patients with severe asthma.

subjects (Table III and Fig 1), with geometric mean levels of 31.4 pg/mL EBC (95% CI, 23.58-41.81 pg/mL EBC) and 2.41 pg/mL EBC (95% CI, 1.31-4.41 pg/mL EBC), respectively (*P* < .001). The difference remained significant after adjustment for age, sex, race, aspirin sensitivity, and use of montelukast. A cutoff value of 7 pg/mL EBC provided 90% sensitivity and 92% specificity for the diagnosis of asthma. The area under the receiver operating curve was 0.96, with a *P* value of less than .001. Low lipoxin levels correlated with decreased lung function (*r* = 0.28, *P* = .018, Fig 2). Among the 3 groups of asthmatic patients, the mean EBC LXA₄ level was numerically lowest in the group with severe asthma (23.54 pg/mL EBC; 95% CI, 16.9-32.78 pg/mL EBC).

EBC LTB₄ levels

The geometric mean of EBC LTB₄ levels in the asthmatic patients was more than 10-fold higher than that in the healthy control subjects (45.62 pg/mL EBC [95% CI, 38.18-54.50 pg/mL EBC] vs 3.82 pg/mL EBC [95% CI, 2.30-6.35 pg/mL EBC], respectively; *P* < .001; Table III and Fig 3). The significance was unaffected after adjustment for age, sex, race, aspirin sensitivity, and montelukast use. There was no overlap in EBC LTB₄ levels

between the asthmatic patients and healthy control subjects. An LTB₄ cutoff value of 11 pg/mL EBC provided 100% sensitivity and 100% specificity for the diagnosis of asthma (area under the curve, 1; *P* < .001). Differences in means among the 3 groups of asthmatic patients were not significant.

EBC CysLT levels

Although all EBC samples were concentrated, CysLT levels were less than the limit of detection in 62% of the samples and thus could not be reliably interpreted.

Ratio between EBC LXA₄ and leukotriene levels

Because more than half of the measured CysLT values were less than the adjusted lower limit of detection, we examined the ratio of EBC LXA₄/LTB₄ levels (Table III and Fig 4). The geometric means for the ratio in healthy control subjects and patients with mild, moderate, and severe asthma were 0.85, 1.13, 0.88, and 0.52, respectively. The ratio was significantly lower in the patients with severe asthma than in those with moderate asthma (0.52 [95% CI, 0.37-0.71] vs 0.88 [95% CI, 0.60-1.27], *P* = .034).

TABLE III. Comparison of LXA₄ and LTB₄ levels and their ratio between EBC obtained from healthy control subjects and participants with mild, moderate, and severe asthma

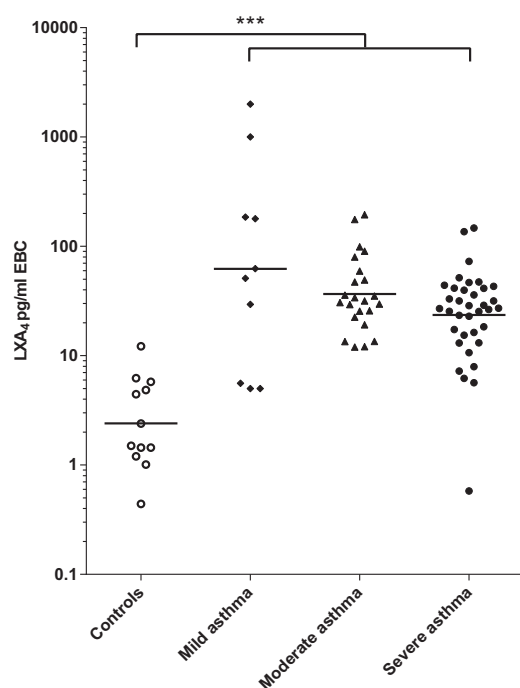
	Healthy control subjects	All asthmatic patients	Patients with mild asthma	Patients with moderate asthma	Patients with severe asthma
No.	12	69	10	23	36
LXA ₄ (pg/mL EBC)	2.41 (1.31-4.41)	31.4* (23.58-41.81)	62.22 (13.48-287.3)	36.61 (26.06-51.43)	23.54 (16.9-32.78)
LTB ₄ (pg/mL EBC)	3.82‡ (2.30-6.35)	45.62* (38.18-54.50)	55.16 (34.10-89.23)	41.81 (31.74-55.08)	45.75 (34.62-60.45)
EBC LXA ₄ /LTB ₄ ratio	0.85‡ (0.34-2.13)	0.69 (0.52-0.92)	1.13 (0.23-5.49)	0.88 (0.60-1.27)	0.52† (0.37-0.71)

Data are presented as geometric means (95% CIs of geometric means). Log-transformed data were analyzed by means of ANOVA and analysis of covariance (adjusted for age, sex, and race). None of the covariates were found to change the parameter estimate or the *P* value of the outcome obtained before adjustment. The *P* value for the difference among the 4 groups for both LXA₄ and LTB₄ values was less than .001.

**P* < .001 for the difference in geometric means of EBC LXA₄ levels between healthy control subjects and asthmatic patients when log-transformed values are compared by using the Student *t* test.

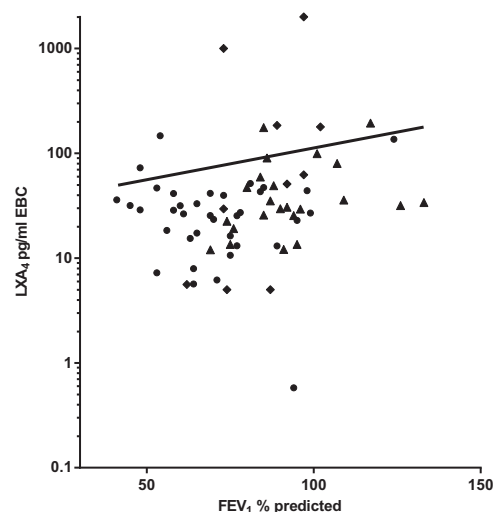
†*P* = .034 for the difference in geometric means between participants with moderate asthma and those with severe asthma when log-transformed values are compared by using the Student *t* test.

‡*N* = 8 because EBC was not available for measurement of LTB₄ levels from 4 healthy control subjects.

**FIG 1.** LXA₄ levels measured in EBC collected from healthy control subjects and participants with mild, moderate, and severe asthma. The sample size is 12, 10, 23, and 36 in each group, respectively. EBC LXA₄ values are plotted on a logarithmic scale on the y-axis. Horizontal bars represent geometric means. ****P* < .001 for the difference in geometric means of EBC LXA₄ levels between healthy control subjects (*n* = 12) and all asthmatic patients (*n* = 69) when log-transformed values are compared by means of ANOVA.

FENO levels

FENO values were available from 22 patients with moderate and 31 with severe asthma. The mean values were 22.74 ± 14.27 and 28.53 ± 23.79 ppb, respectively (Table II). Sixty-eight percent of the participants with moderate asthma and 39% of the participants with severe asthma in whom such measurements were made had FENO values of greater than 25 ppb (the suggested upper value for normal).²⁵ In the same population 100% of patients with moderate asthma and 90% of patients with severe asthma had an LXA₄ level of greater than 7 pg/mL EBC. All of these 53 asthmatic patients had LTB₄ levels of greater than 11 pg/mL EBC.

**FIG 2.** Correlation between EBC LXA₄ levels and airflow obstruction measured as FEV₁ percent predicted. Diamonds, Patients with mild asthma (*n* = 10); triangles, patients with moderate asthma (*n* = 23); circles, patients with severe asthma (*n* = 36). *r* = 0.28 and *P* = .018 by Spearman correlation.

DISCUSSION

Using direct *in vivo* measurements from the airways, we demonstrate that the lipoxin and leukotriene pathways are upregulated in patients with asthma of all severities. Our data also provide *in vivo* confirmation that relative underproduction of lipoxins occurs with increasing asthma severity. Lastly, our data suggest that noninvasively obtained EBC measurements of representative eicosanoids from these pathways distinguish asthmatic from non-asthmatic patients with high sensitivity and specificity. These results suggest a pathobiologic role for counterregulatory eicosanoids in addition to proinflammatory eicosanoids in asthmatic patients. They further suggest that noninvasively measured eicosanoids can serve as biomarkers for asthmatic airway diathesis and its severity.

Lipoxins and leukotrienes are biologically active products derived from arachidonic acid with distinct structure and function. Lipoxins are produced by airway structural cells, macrophages, and leukocytes during inflammatory responses.²⁶ They exert potent anti-inflammatory and proresolving effects and reduce hyperresponsiveness in the airways of patients with asthma, thus functioning as counterregulatory molecules that maintain

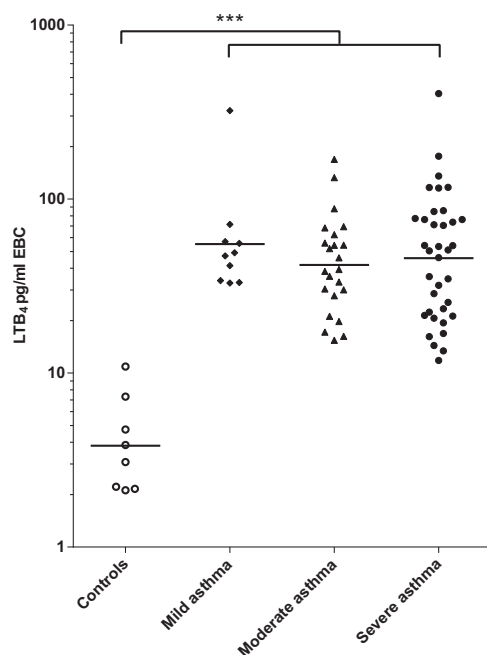


FIG 3. LTB₄ levels measured in EBC collected from healthy control subjects and participants with mild, moderate, and severe asthma. The sample size is 8, 10, 23, and 36 in each group, respectively. Measured EBC LTB₄ values are plotted on a logarithmic scale on the y-axis. Horizontal bars represent geometric means. ****P* < .001 for the difference in geometric means of EBC LTB₄ levels between healthy control subjects (*n* = 8) and all asthmatic patients (*n* = 69) when log-transformed values are compared by means of ANOVA.

tissue homeostasis.^{3,4} On the other hand, leukotrienes are potent chemoattractants and activators of inflammatory cells,² the levels of which are increased in sputum, plasma, BAL fluid, and EBC from asthmatic patients. In addition, they are postulated to play a role in the pathobiology of the disease.^{19,27-29} We sought to examine the potential utility of noninvasively obtained measurements of these 2 countervailing families of eicosanoids as biomarkers for asthma and its severity. We were unable to obtain measurements of CysLTs from more than half of the samples examined; however, we found that we could easily measure LXA₄ and LTB₄ levels in EBC. Our data show that compared with healthy control subjects, both proresolving and proinflammatory pathways, as represented by EBC LXA₄ and LTB₄ levels, respectively, are upregulated nearly 10-fold in asthmatic patients.

We found EBC LXA₄ levels to be increased in patients with all severities of asthma, including those with mild disease. Considering the broad proresolving function played by these eicosanoids as they relate to the proinflammatory processes that occur in asthmatic patients, the upregulation of this pathway is not surprising. However, Fritscher et al²⁰ recently found that EBC LXA₄ levels in patients with mild asthma are no different than those in healthy control subjects. One explanation for these conflicting results could be that the group of patients with mild asthma examined by them were phenotypically different from those examined by us and other groups. Our data with regard to increased EBC LXA₄ levels in patients with mild asthma are consistent with findings reported in blood obtained from a pediatric cohort by Wu et al¹⁴ and in sputum from adults in 2 prior reports.^{12,16}

We identified an association between reduced EBC LXA₄ levels and decreasing lung function. Our findings related to the

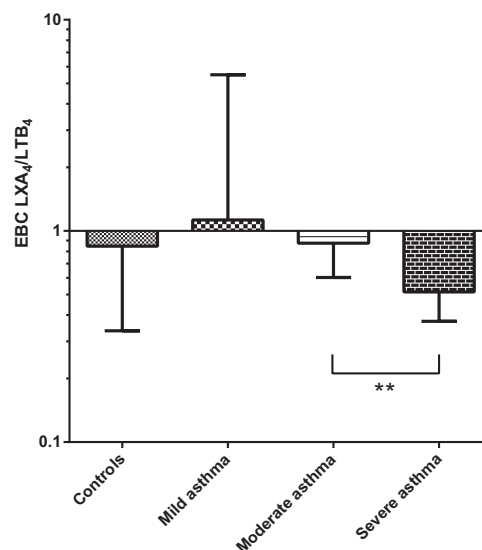


FIG 4. Ratio of EBC LXA₄ levels to EBC LTB₄ levels in healthy control subjects and participants with mild, moderate, and severe asthma. The sample size is 8, 10, 23, and 36 in each group, respectively. Column bars represent the geometric mean of EBC LXA₄/LTB₄ values plotted on a logarithmic scale on the y-axis. Vertical lines represent the 95% CI of the geometric mean. ***P* = .034 for the difference in geometric means of EBC LXA₄/LTB₄ values between patients with moderate asthma (*n* = 23) and patients with severe asthma (*n* = 36) when log-transformed values are compared by using the Student *t* test.

relative production of LXA₄ compared with LTB₄ are of particular interest. We found that although both LXA₄ and LTB₄ levels were increased in the airways of asthmatic patients (Table III and Figs 1 and 3), the ratio between the 2 decreased with increasing asthma severity (Table III and Fig 4). The mean LXA₄/LTB₄ ratio was 41% lower in the patients with severe asthma when compared with that in patients with moderate asthma (*P* = .034).

These findings regarding LXA₄ in EBC support data suggesting that lipoxin biosynthetic capacity might be defective in patients with severe asthma and thus contribute to the perpetuation of airway inflammation in these patients. They are also consistent with those of Vachier et al¹⁶ in induced sputum, who noted decreased sputum lipoxin levels with increasing asthma severity, and with those of Fritscher et al²⁰ in EBC. Furthermore, recent data also suggest that these counterregulatory pathways might be impaired in asthma exacerbations.^{21,30} Altogether, these data suggest that the proresolving pathways not only play a role in the pathophysiology of asthma but also that defects in this pathway might be associated with increasing asthma severity.

Patients with severe asthma are treated with significantly higher doses of ICSs compared with those with less severe disease. We have observed that LPS-induced formation of LXA₄ by alveolar macrophages is decreased by dexamethasone in both patients with nonsevere and those with severe asthma *ex vivo*.³¹ It is thus possible that our observations in patients with severe asthma relate to the presence and amounts of ICSs rather than the intrinsic disease process. However, we did not find a relationship between inhaled or oral corticosteroid doses and LXA₄ levels in BAL fluid obtained from subjects with severe asthma.¹¹ Furthermore, Hasan et al²¹ did not see a change in EBC LXA₄ levels after 24 hours of systemic glucocorticoid therapy in children who presented with status asthmaticus. Finally, although mean EBC

LXA₄ levels in our cohort were lower in the patients with severe asthma receiving high doses of ICSs than in the patients with mild intermittent asthma not using ICSs, they were in fact lowest in the healthy control subjects. This argues against the effects of steroids on arachidonic acid metabolism leading to biased study results.

We found that exhaled LTB₄ levels were more than 10 times higher in asthmatic patients compared with those in nonasthmatic patients, without any overlap in levels between the 2 groups. These findings are consistent with upregulation of the leukotriene eicosanoid pathway in asthmatic patients and consistent with the findings of others. High LTB₄ levels have been reported in blood samples from a pediatric asthmatic cohort when compared with healthy control subjects.¹⁴ Vachier et al¹⁶ also noted an increase in LTB₄ levels in induced sputum in asthmatic patients versus control subjects. However, their results did not reach statistical significance, possibly related to their small sample size. All studies that have examined EBC LTB₄ report increased levels in patients with moderate-to-severe asthma when compared with control subjects.^{20,32,33} However, they differ in their findings in patients with mild asthma. In 2 studies LTB₄ levels in asthmatic patients not receiving controller therapy were increased,^{29,32} whereas in another study they were not.²⁰ In a fourth study EBC LTB₄ levels were increased in patients with mild persistent asthma but not in patients with mild intermittent asthma.³³ Although our data are more consistent with the former studies, the discrepancies suggest that further phenotyping might be necessary to ascertain the potential causes for these observed differences.

EBC collection is completely noninvasive; the equipment is technologically simple and low cost and requires minimal operator training.^{18,19} Hence it is informative to compare our findings with FENO values, the only other exhaled biomarker that has been accepted as a useful tool in the noninvasive assessment of asthma. Values of less than 25 ppb can reliably rule out eosinophilic asthmatic airway inflammation.²⁵ However, it has now become increasingly clear that a subgroup of patients with severe asthma have paucieosinophilic airway inflammation, which not only makes them refractory to corticosteroid therapy but also makes them undistinguishable from healthy control subjects and other asthmatic patients when using the FENO cutoff value.³⁴ Moreover, the utility of FENO measurement in patients on ICSs is quite limited because of the profound suppression of FENO by glucocorticoids, even at low doses.³⁵ If the threshold of 25 ppb for FENO values were to be applied to our cohort, 32% of patients with moderate and 61% of those with severe asthma would fall below that threshold. In contrast, almost none of them would have been misclassified if LXA₄ thresholds of 7 pg/mL EBC and LTB₄ thresholds of less than 11 pg/mL EBC were applied.

Although our findings suggest that EBC LXA₄ and LTB₄ might be useful biomarkers for asthma and its severity, several caveats do need to be considered. Although we observed increased LXA₄ and LTB₄ levels in patients with mild asthma, as discussed above, findings in patients with intermittent asthma by others have been inconsistent.^{20,29,32,33} Whether these differences relate to phenotypic differences in the populations is unclear, and studies in large well-phenotyped cohorts will be required to clarify these observations. Furthermore, it should be noted that increased eicosanoid levels are not restricted to asthma. Studies have noted increased levels of LXA₄ and LTB₄ in blood and respiratory samples of patients with other

inflammatory conditions, such as chronic obstructive lung disease, cystic fibrosis, and scleroderma lung disease.^{16,20,36-39} These data suggest that proresolving eicosanoid responses are not specific to any one particular inflammatory airway disease but represent the host's defense mechanisms to counteract eicosanoid-mediated airway inflammation. We propose that EBC LXA₄ and LTB₄ levels serve as noninvasive biomarkers for the presence of airway inflammation in asthmatic patients, identify dysregulated eicosanoid patterns in those with severe disease, and thus supplement the information obtained by means of clinical assessment of patients by their physicians.

In summary, our data show that both inflammatory and proresolving eicosanoid pathways are upregulated in asthmatic airways. Furthermore, our *in vivo* measurements indicate that relative underproduction of lipoxins occurs in the airway with increasing asthma severity. These results provide insight into the pathobiological role of lipid mediator-driven airway inflammation in asthmatic patients and suggest that interventions to increase proresolving pathways might be of importance in treating severe asthma. Although they require confirmation in larger cohorts, these data also suggest that noninvasive EBC measurements can be used as sensitive and specific biomarkers to distinguish asthma from nonasthma, especially in patients with more severe disease, in whom other tests might have less utility.

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Clinical implications: Exhaled LPA₄ and LTB₄ measurements can be used as noninvasive biomarkers for the diagnosis of asthma and assessment of its severity. Interventions to increase proresolving lipoxins might be of importance in treating severe asthma.

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