

Establishing Urinary Leukotriene E₄ as a Diagnostic Biomarker for Chronic Rhinosinusitis with Comorbid Asthma and Atopy

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Abstract

Objective. While urinary leukotriene E₄ (uLTE₄) is a validated biomarker for the cysteinyl leukotriene pathway, which is central to the pathophysiology of asthma, atopy, and chronic rhinosinusitis (CRS), the contributions of comorbid asthma and atopy to uLTE₄ levels in various CRS subtypes have not been previously characterized. We sought to (1) identify reference values for uLTE₄ in subjects with and without CRS and (2) determine how the presence of comorbid atopy and asthma affects uLTE₄ levels in CRS.

Setting. Tertiary referral medical center.

Subjects and Methods. A prospective case-control study was conducted to compare uLTE₄ levels between patients with CRS and healthy controls. Urinary LTE₄ levels were measured by enzyme immunoassay and were adjusted for urinary creatinine concentrations (pg/mg Cr). Patients with CRS were stratified by the clinical comorbidities to determine normative uLTE₄ values for patients with CRS with and without comorbid asthma or atopy.

Results. A total of 153 patients (mean age, 47.3; 47.1% female) were included in the study. Patients with CRS demonstrated significantly higher concentrations of uLTE₄ than healthy controls (1652 vs 1065 pg/mg Cr, *P* = .032). Within the group of patients with CRS, comorbid asthma also individually correlated with elevated uLTE₄ levels (1597 pg/mg Cr, *P* = .0098). Patients with CRS who did not have comorbid allergy and asthma, in contrast, did not have statistically higher uLTE₄ levels than healthy controls (1142 pg/mg Cr, *P* = .61).

Conclusion. Urinary LTE₄ serves as a noninvasive measure of the inflammatory state in CRS. Comorbid asthma and atopy contribute to elevated uLTE₄ levels in CRS.

Keywords

chronic sinusitis, asthma, allergy, leukotriene, biomarker

Cysteinyl leukotrienes (CysLTs) are multifunctional lipid mediators that are synthesized from arachidonic acid via the 5-lipoxygenase pathway (5-LO) (Figure 1) and participate in systemic inflammatory reactions.¹ Mast cells, basophils, eosinophils, and macrophages facilitate the conversion of arachidonic acid to the most stable end product, leukotriene E₄ (LTE₄).² LTE₄ is regarded as a biomarker for total-body CysLTs and is excreted in the urine.¹ Measuring urinary LTE₄ levels (uLTE₄) has previously been shown to be a noninvasive, sensitive method of assaying total-body CysLT production.

LTE₄ has been shown to be a reliable biomarker in inflammatory states such as asthma,³ allergic rhinitis,⁴ and aspirin sensitivity.⁵ Chronic rhinosinusitis (CRS) is a complex, multifactorial inflammatory process in which the role of leukotrienes is less defined. Previous studies have shown uLTE₄ levels to best correlate with CRS associated with aspirin sensitivity.⁶ Aspirin-exacerbated respiratory disease (AERD) is driven by proinflammatory cytokines that exert their effects on vascular smooth muscle and mucosal epithelium, leading to both pulmonary and systemic vasoconstriction, increased vascular permeability, mucus hypersecretion, edema, and inflammatory cell activation.^{1,7} The systemic expression of LTE₄ in CRS subtypes other than AERD is still evolving, but it is presumed that comorbid asthma and allergies influence the inflammatory cytokine profile in CRS. The heterogeneity of CRS at a biomarker level is driven by adaptive immune changes and allergic priming, which may contribute to increased levels of leukotrienes and sinonasal inflammation.⁸

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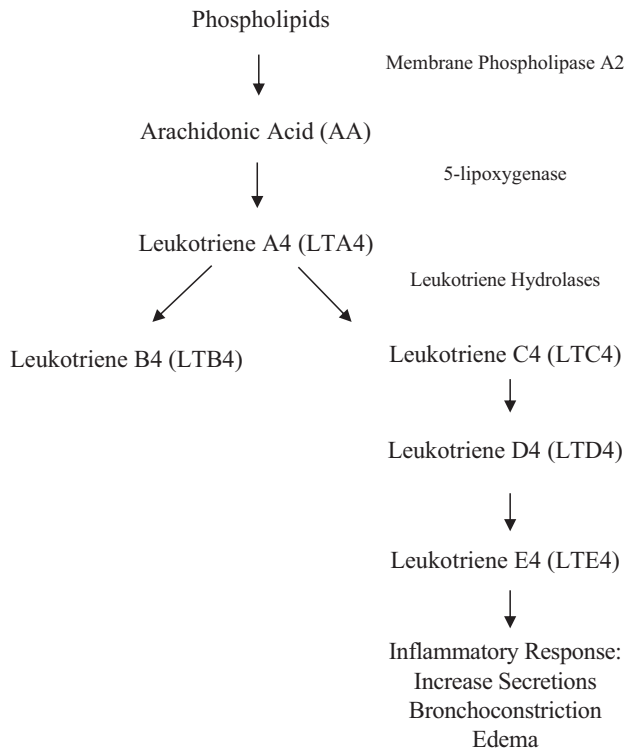


Figure 1. Cysteinyl leukotriene (CysLT) pathway. Leukotriene E_4 serves as a stable end product of the metabolic pathway that converts arachidonic acid into a variety of proinflammatory mediators.

The current study investigates the variation in $uLTE_4$ levels in patients with CRS, asthma, and allergies using a validated immunoassay on a spot urine specimen. Given the potential contributions of asthma and allergies to overexpression of LTE_4 , we intend to identify CRS subgroups that experience elevated $uLTE_4$ levels. The 2 goals of the study are to (1) identify reference values for $uLTE_4$ in subjects with and without CRS and (2) determine how the presence of comorbid atopy and asthma affects $uLTE_4$ levels in CRS. CRS diagnostic and treatment algorithms vary based on comorbid conditions, and improved methods to classify CRS with noninvasive biomarkers can allow for better individualization of care.

Materials and Methods

Study Group

Institutional review board approval was obtained from Eastern Virginia Medical School for this prospective case-control study. The study duration was between January 2016 and January 2017. All study participants, who were between the ages of 7 and 70, were selected during routine evaluation in the rhinology practice of a single tertiary care center. Written informed consent was obtained from all subjects prior to their study enrollment and 1-time collection of a urine specimen.

Patients with CRS were included in the study if they demonstrated a positive diagnosis of CRS by symptomatology

based on the American Academy of Otolaryngology–Head and Neck Surgery (AAO-HNS) CRS criteria and had supporting endoscopic or computed tomography (CT) findings. Patients with CRS with and without nasal polyps were included in this study. Patients with CT evidence of disease had a Lund-Mackay score of at least 4. Comorbid asthma and atopy were ascertained from the patient intake form. The comorbid diagnosis of asthma was thereafter confirmed by a pulmonologist with a positive bronchoprovocation spirometry testing. Pulmonology evaluation was limited to patients with respiratory symptoms, patients with a previous asthma diagnosis, or those who previously underwent respiratory-related treatment. Atopy was confirmed by clinical history and accompanying skin or blood testing. Allergy testing was included for all patients in the study.

Patients with CRS were excluded from the study if they had a coexisting immunologic compromise, immunodeficiency, or corticosteroid-dependent condition. Patients with CRS were also excluded from the study if they had taken systemic corticosteroids 4 weeks prior to study enrollment, were using nasal corticosteroid drops or rinses, or were taking any leukotriene-modifying medication, such as zileuton or montelukast. Traditional intranasal steroid medications were not a point of exclusion because of their ubiquity in the referral population.

The control group did not have any clinical, endoscopic, or radiographic evidence of CRS. Control subjects were patients who completed the prescreening process for complaints that were not related to CRS, such as nasal septum deviation, pituitary tumors, and hoarseness. Controls were excluded from the study if they had an accompanying inflammatory or infectious medical condition that could alter $uLTE_4$ levels.

Urinary Leukotriene E_4 Levels

Spot urine samples were collected from all study participants during their office encounters. Approximately 10 mL of urine was saved and immediately stored in a -80°C ultralow Freezer (Fischer Scientific, Waltham, Massachusetts). An enzyme-linked immunosorbent assay was then used to detect LTE_4 levels (Cayman Chemical, Ann Arbor, Michigan). All specimens were run in duplicate. Baseline creatinine levels were also calculated from the same urine samples, and the $uLTE_4$ levels were adjusted accordingly to urinary creatinine values. Values were reported as pg/mg Cr.

Statistical Analysis

Summary statistics were calculated to describe the sample characteristics. A Student t test and analysis of variance (ANOVA) were used to compare baseline characteristics. A Student t test was also used to compare $uLTE_4$ levels between patients with CRS and controls. Asthma and allergies were recorded. Two-sample t test and χ^2 test were used to detect the differences between the 2 groups for continuous outcomes and categorical outcomes, respectively. ANOVA was used to compare continuous measurements for more than

Table 1. Baseline Patient Characteristics of Samples Collected for Study.

Characteristic	Total	
Patient characteristics		
Total No. of patients	153	
Age, mean (range), y	7-70 (47.3)	
Sex: female, No. (%)	72 (47.1)	
CRS patients, No. (%)	115 (75.1)	
Control patients, No. (%)	38 (24.9)	
CRS subclassification, No. (%)		
CRS patients	115 (75.1)	
CRS + asthma	4 (2.6)	
CRS + allergy	48 (31.3)	
CRS + asthma + allergy	41 (26.8)	
CRS without asthma/allergy	22 (14.4)	
Characteristic Comparison	CRS (n = 115)	Control (n = 38)
Age range (mean), y	7-70 (47.3)	25-70 (47.4)
Sex: female, No. (%)	50 (43.3)	22 (57.9)

Abbreviation: CRS, chronic rhinosinusitis.

Table 2. Urinary Leukotriene Levels Distributed by Subgroup.^a

Condition	Patient Count	Urinary Leukotriene E ₄ Average (pg/mg Cr), mean ± SD (95% CI)
Total No.	153 (100)	1519.1 ± 1032.5
Control	38 (24.9)	1065.1 ± 473.2 (591.9-1583.3)
CRS	115 (75.1)	1652.7 ± 1118.3 (534.4-2771.1)
CRS, no asthma/allergy	22 (14.4)	1142.8 ± 599.4 (543.3-1742.2)
CRS + allergy	48 (31.3)	1456.9 ± 1324.4 (132.5-2781.3)
CRS + asthma	4 (2.6)	1597.1 ± 838.4 (758.7-2435.6)
CRS + asthma + allergy	41 (26.8)	1874.2 ± 968.2 (906.0-2842.3)

Abbreviations: CI, confidence interval; CRS, chronic rhinosinusitis.

^aPatients within the CRS group were subcategorized in to 4 cohorts dependent on their allergy and asthma status.

2 groups. A *P* value of less than .05 was considered statistically significant. All data analyses were performed in SAS 9.4 (SAS Institute, Cary, North Carolina).

Results

Baseline Characteristics

A total of 153 patients were included in the study. All 153 patients within the CRS and control groups provided urine samples (**Table 1**). The study group included 115 patients with CRS and 38 control patients. Seventy-two patients were female. Forty-four patients had asthma, and 89 had allergies. Twenty-two patients had CRS but did not have asthma or allergies. The average age for the CRS population was 47.3 years, and females accounted for 43.3% of the CRS cohort. For the control group, the average age was 47.4 years, and 57.9% of the control cohort were female. There were no statistically significant differences between age and sex in the

CRS and control populations. There was a nonequal distribution of patients in further subgroup analysis.

Urinary Leukotriene E₄ (uLTE₄) Levels in CRS

Urinary LTE₄ levels were significantly higher in patients with CRS (1652 pg/mg Cr) compared to healthy controls (1065 pg/mg Cr; *P* = .032) (**Tables 2** and **3**). Within the cohort of patients with CRS, comorbid asthma and atopy separately demonstrated elevated uLTE₄ levels. CRS with asthma had significantly elevated uLTE₄ relative to controls (1597 pg/mg Cr; *P* = .0098). CRS with allergies had elevated levels of uLTE₄ but did not meet statistical significance (1456 pg/mg Cr; *P* = .143). Patients with CRS who had both asthma and allergy also had elevated uLTE₄ levels relative to controls (1874 pg/mg Cr; *P* = .00008), accounting for the highest uLTE₄ levels in the study. Patients with CRS who did not have comorbid allergy and asthma, in

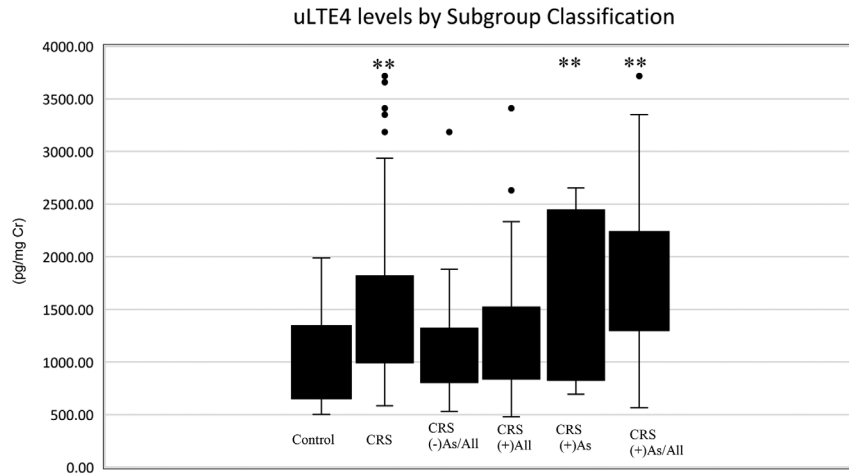


Figure 2. Subgroup analysis of urinary leukotriene E₄ (uLTE₄) levels. This box graph highlights the uLTE₄ levels, presented in units of pg/mg Cr, for the healthy control group and the different chronic rhinosinusitis (CRS) subgroups based on the presence or absence of comorbid asthma or allergy.

Table 3. Analysis with Comparison of CRS Subgroups and uLTE₄ Levels.^a

Diagnosis	Comparison to Control Group: ANOVA P Value
CRS	.032 ^b
CRS, no asthma/allergy	.61
CRS + allergy	.143
CRS + asthma	.0098 ^b
CRS + asthma + allergy	.00008 ^b

Abbreviations: ANOVA, analysis of variance; CRS, chronic rhinosinusitis.

^aThe urinary leukotriene E₄ (uLTE₄) levels in patients with CRS who had comorbid asthma and allergies were compared with the control group to determine statistical significance.

^bDenotes statistical significance ($P < .05$).

contrast, did not have statistically higher uLTE₄ than healthy controls (1142 pg/mg Cr; $P = .61$). We subsequently compared the uLTE₄ levels between patients with CRS with asthma and patients with CRS with allergy and were unable to detect a difference with multivariate analysis that suggested asthma or allergy was a greater contributing factor to uLTE₄ elevation ($P = .36$). **Figure 2** displays the uLTE₄ levels for the healthy control and CRS subgroups as classified by the presence or absence of comorbid asthma and allergy.

A total of 11 patients with a diagnosis of AERD were also included in the study. Because by definition, AERD patients have coexisting asthma, these 11 AERD patients were included in the previously mentioned groups as part of the subgroup analysis. However, when isolated and evaluated as a singular group, the AERD patient cohort had a uLTE₄ level of 1572 pg/mg Cr.

Discussion

Urinary LTE₄ is an emerging biomarker for inflammatory pathways in CRS. Enzyme immunoassays have been

established as a sensitive method for measuring uLTE₄ levels in crude spot urine specimens since 1994.⁹ Asthma³ and allergy⁴ cohorts in isolation have previously been the most studied for variation in uLTE₄ levels. There has been little to date assessing contributions of comorbid asthma and allergy responses in CRS. This is the first prospective study to show that asthma correlated with elevated uLTE₄ levels in CRS ($P = .0098$) while an allergy profile did not show statistical significance for uLTE₄ levels ($P = .143$).

In our study, patients with CRS with asthma mounted a larger uLTE₄ response than control patients (1597 vs 1065 pg/mg Cr; $P = .0098$). Our study cohort included all asthmatic phenotypes, including AERD. Previous leukotriene studies in asthma have shown increased uLTE₄ with early allergen-induced bronchoconstriction and increased in infants with atopic/asthmatic backgrounds.¹⁰ There is variability in asthma endotypic expression of leukotrienes, but aspirin-sensitive asthmatics have been the most consistently observed to exhibit increases in uLTE₄ levels.⁵ Lung respiratory epithelium has specialized compartments for eicosanoid biosynthesis,¹¹ and respiratory disorders are thought to disrupt epithelial tight junctions, causing local changes in eicosanoid concentrations.¹² The activation of leukotriene pathways in acute asthma attacks has been shown to correlate with the degree of airflow obstruction with uLTE₄ levels, causing a reflexive decrease in forced expiratory volumes.¹³

Patients with CRS with allergies alone mounted a larger uLTE₄ response than controls, but this difference did not achieve statistical significance (1456 pg/mg Cr; $P = .143$). The hypersensitivity exhibited in allergy and asthma is not a dichotomous, isolated process but rather a continuum of shared inflammatory pathways involving similar effector cells. Previous immunostaining studies have shown overexpression of CysLT receptors along the epithelium of the inferior turbinates in patients with nasal allergies.¹⁴ Similar studies have shown that CysLTs play a significant role in allergic priming by upregulating allergen-specific dendritic cells to increase production

of interleukins, class switching, and recruitment of inflammatory cells to the sinonasal epithelium.¹⁵

Asthma and allergy in patients with CRS concurrently contributed to the highest uLTE₄ levels (1874 mg/pg Cr; $P = .00008$). The hyperleukotrienemia exhibited in patients with CRS with both asthma and allergies could be secondary to greater crossover between shared inflammatory pathways. Recent reviews have highlighted the shared pathophysiology between asthma and allergy.¹⁶ The concept of “one airway, one disease” in regards to nasobronchial inflammation is reinforced by the findings of our study. Our study shows a correlation with asthma and allergies in CRS but did not evaluate shared upstream contributing biochemical pathways. This is an area that requires further investigation.

In our study, CRS without asthma and allergy subgroup analysis did not show a difference in uLTE₄ levels relative to controls (1142 pg/mg Cr; $P = .61$). Patients with CRS who lack an asthmatic and allergic component are thought to have a more type 1 inflammatory response with proinflammatory pathways less likely to include IgE responses.¹⁷ A more innate immune response, which is less dependent on leukotriene upregulation, would be expected to occur in isolated CRS as reflected in our study.

While there is variability in collection methods for uLTE₄, our institution used a spot urine specimen collection that was processed through an immunoassay technique. Multiple studies have shown reliable results using spot urine specimens to measure leukotriene levels. A recent 2016 study evaluating a new method for uLTE₄ analysis compared the distribution of uLTE₄ concentrations in 24-hour and random urine collections and found no difference in uLTE₄ levels based on length of collection.¹⁸ Specimens were also collected throughout the day in clinic as patients presented for consultation. No preference was given to the time of collection because specific diurnal variation patterns for uLTE₄ levels have not been described previously.³

When comparing analysis methods for urine leukotriene specimens, there is heterogeneity in the literature for ranges of acceptable values. Previous sampling methods have included enzyme immunoassay, mass spectrometry, and radioimmunoassay. When comparing all methods in a systematic review, Hagan et al¹⁹ determined that the diagnostic odds ratio for each method of analysis is variable and that a “standardized mean” for uLTE₄ levels is currently lacking. While our control level of 1065 pg/mg Cr is elevated relative to previous values, including those by Asano et al,³ our trends in the subgroup analysis for the patients with CRS reflect the importance of increasing values relative to the concomitant inflammatory burden. Recent work in pediatrics²⁰ has found a more similar mean uLTE₄ level of 803.4 pg/mg Cr, which is reported as 91.3 ng/mM by the study group, in patients evaluated for sleep-disordered breathing. There is variability in the field currently with introducing uLTE₄ as a clinically useful biomarker. Additional work is thus required to standardize the methods of acquisition and analysis of specimens for uLTE₄ levels.

In regard to study limitations, patients included in the study were recruited and enrolled at a tertiary health center,

where more medically complex patients with CRS are evaluated. Therefore, a bias may exist toward elevated uLTE₄ levels because of a potentially larger inflammatory burden. Second, a number of confounding variables related to patient characteristics, including smoking status, obesity, and recent acute viral illnesses, deserve mention. While these confounding variables undoubtedly affect the uLTE₄ levels measured in this study, they improve the generalizability of the study results in the real-world setting in which a myriad of patient factors ultimately influence the clinical outcomes imparted by different CRS interventions. A third limitation of this study is reflected in the fact that the uLTE₄ enzyme immunoassay is an advanced, expensive test. Urinary LTE₄ is not a reliable in-office assay at this time. Therefore, it is difficult to speculate on its clinical applicability in future practice.

Conclusion

Elevated uLTE₄ levels are a global indicator of inflammatory burden in CRS. In this study, we determined that both allergy and asthma contribute to significant increases in uLTE₄ levels in CRS. CRS without comorbid allergy and asthma did not correlate with elevated uLTE₄ levels. Measuring uLTE₄ thus provides a noninvasive approach to identify CRS subtypes in which sinonasal inflammation is modulated by the CysLT pathway and to offer potential therapies that are targeted to the underlying pathophysiologic processes.

Author Contributions

Griffin D. Santarelli, design, data collection, analysis, and revisions; **Kent K. Lam**, design, data collection, analysis, and revisions; **Joseph K. Han**, design, data collection, analysis, and revisions.

Disclosures

Competing interests: None.

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