Original Article

Diagnostic Utility of Urinary LTE4 in Asthma, Allergic Rhinitis, Chronic Rhinosinusitis, Nasal Polyps, and Aspirin Sensitivity

Rohit Divekar, MBBS, PhD[a], John Hagan, MD[a], Matthew Rank, MD[b], Miguel Park, MD[a], Gerald Volcheck, MD[a], Erin O'Brien, MD[c], Jeffrey Meeusen, PhD[d], Hirohito Kita, MD[a], and Joseph Butterfield, MD[a] *Rochester, Minn; and Scottsdale, Ariz*

What is already known about this topic? Urinary leukotriene E4 (LTE4) is a well-validated marker of the cysteinyl leukotriene pathway, and LTE4 elevation has been described in conditions such as asthma, aspirin sensitivity, and chronic rhinosinusitis (CRS).

What does this article add to our knowledge? This study explores the diagnostic utility of 24-hour urinary LTE4 in 5 clinical diagnoses of allergic rhinitis, asthma, CRS with/without nasal polyps, and aspirin sensitivity. Elevations in LTE4 were seen in asthma and CRS with nasal polyps but influenced by underlying aspirin sensitivity.

How does this study impact current management guidelines? A cutoff of 166 pg/mg Cr suggests the presence of history of aspirin sensitivity with 89% specificity (useful, if history is unclear), and a cutoff of 241 pg/mg Cr could discriminate challenge-confirmed aspirin sensitivity with 92% specificity.

BACKGROUND: Urinary leukotriene E4 (LTE4) is a well-validated marker of the cysteinyl leukotriene pathway, and LTE4 elevation has been described in conditions such as asthma, aspirin sensitivity, and chronic rhinosinusitis (CRS). There have been a number of reports investigating the role of spot urine LTE4 to predict aspirin sensitivity; however, variability in urinary LTE4 may affect the accuracy of this approach.

OBJECTIVE: Here, we explored the utility of 24-hour urinary LTE4 in 5 clinical diagnoses of allergic rhinitis, asthma, chronic rhinosinusitis with nasal polyps (CRSwNP), CRS without nasal polyps, and aspirin sensitivity.

METHODS: This was a *retrospective* review of patients who had 24-hour quantification of urinary LTE4 by a clinically validated liquid chromatography tandem mass spectrometry method and their assigned diagnoses after assessment and clinical care.

RESULTS: Twenty-four-hour urinary LTE4 elevations were seen in those with asthma and those with CRSwNP but influenced by underlying aspirin sensitivity. Elevation in LTE4 was significant in those with CRSwNP after adjusting for aspirin sensitivity. Allergic rhinitis was not associated with elevated LTE4 excretion. Receiver operator characteristic analysis of 24-hour urinary LTE4 showed that a cutoff value of 166 pg/mg Cr suggested the presence of history of aspirin sensitivity with 89% specificity, whereas a cutoff value of 241 pg/mg Cr discriminated "challenge-confirmed" aspirin-sensitive subjects with 92% specificity.

CONCLUSIONS: Elevated 24-hour excretion of urinary LTE4 is a reliable and simple test to identify aspirin sensitivity in patients with respiratory diagnoses. © **2016 American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2016;4:665-70)**

Key words: *Urinary; Leukotriene; LTE4; Aspirin; Sensitivity; AERD; Rhinosinusitis; Diagnostic; Specificity; Predictive*

Chronic rhinosinusitis (CRS) is a complex disease entity that stands alone or with associations with atopy, asthma, nasal polyps (NP), and aspirin sensitivity. This heterogeneity is regularly encountered by the practicing allergist due to these comorbid disease states.[1] Aspirin- exacerbated respiratory disease (AERD) is a chronic unrelenting condition characterized by persistent upper airways inflammation including CRS, NP, and/or persistent lower airways inflammation leading to asthma. The disease is seemingly more complex than its simplistic inclusion as a subset of either CRS or severe asthma and presents unique clinical challenges.[1,2] Although exact numbers are difficult to ascertain, one report

[a]Division of Allergic Diseases, Department of Medicine, Mayo Clinic, Rochester, Minn
[b]Division of Allergy, Asthma, and Clinical Immunology, Mayo Clinic, Scottsdale, Ariz
[c]Department of Otorhinolaryngology, Mayo Clinic, Rochester, Minn
[d]Laboratory Medicine and pathology, Mayo Clinic, Rochester, Minn
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Corresponding author: Rohit Divekar, MBBS, PhD, Division of Allergic Diseases, 200 First St SW, Rochester, MN 55905. E-mail: Divekar.Rohit@mayo.edu.
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Abbreviations used
AERD- aspirin exacerbated respiratory disease
AUC- area under the curve
CRS- chronic rhinosinusitis
CRSsNP- chronic rhinosinusitis sans *(without) nasal polyps*
CRSwNP- chronic rhinosinusitis with nasal polyps
DOR- diagnostic odds ratio
LC-MS- liquid chromatography/mass spectrometry
LTE4- leukotriene E4
ROC- receiver operator characteristic
NP- nasal polyps
NPV- negative predictive value
PPV- positive predictive value

using the Centers for Disease Control and Prevention data indicates that 8.2% of the US population has asthma and among adult patients with asthma, up to 9% have AERD.[3] In addition, CRS prevalence was reported to be approximately 13%, and 15% of those CRS patients with NP have AERD.[3] Thus, this overlap can present a diagnostic conundrum. In addition to a distinct and increased symptom burden present in this population,[4,5] this group has an exceptionally high utilization of health care in terms of frequent and complex regimens of medications, oral and topical therapies, multiple sinus surgeries, increased burden of difficult to control asthma, increased frequency of emergency room visits, and inpatient hospitalization for severe asthma.[6,7] Urinary LTE4 is a biomarker of increased cystinyl leukotriene activity; however, the relationship of urinary leukotrienes to particular phenotypes, beyond aspirin sensitivity, has not been adequately addressed.[8] LTE4 is the stable metabolite of LTC4 and LTD4. Sources of LTE4 include many of the cells types involved in inflammation of the upper and lower respiratory tract such as eosinophils, mast cells, basophils as well as macrophages, platelets, and neutrophils.[9,10] In addition, most studies to date documenting an increase in urinary LTE4 in aspirin sensitivity have used an immunoassay. In addition, studies have used urine spot specimen[11-13] rather than 24-hour collection and the reported variability in urinary LTE4 production may influence these findings.[14] In the present study, we performed a "real-world" *retrospective* analysis of the utility of 24-hour urinary LTE4 as measured by clinically validated liquid chromatography/mass spectrometry (LC-MS) in subjects with clinical diagnoses of allergic rhinitis, asthma, CRS with or without NP, and aspirin sensitivity.

METHODS

Study design

This was a *retrospective* study of all subjects who underwent measurement of 24-hour urinary LTE4 at our institution between March 2014 and April 2015. The study was approved by the institutional review board of the Mayo Clinic.

Subjects and diagnosis

All patients who underwent LTE4 testing were selected for analysis from a laboratory list. Patients' charts were reviewed for the diagnoses after completion of care and visit to ensure only final diagnoses were considered for analyses. Patients who were found to have mast cell– related disorders (including mastocytosis), angioedema, or urticaria were excluded from this analysis. Patients with a respiratory-related diagnosis such as allergic rhinitis, asthma, CRS with or without NP, or aspirin sensitivity were included and those who did not have any of the above diagnoses were used as controls for comparison. The controls thus consisted of all patients without the diagnosis of interest and included those patients who underwent evaluation for possible systemic mastocytosis, mast cell activation disorders, urticaria, or angioedema but after workup no specific cause was determined.

TABLE I. Characteristics of subjects in the study

Characteristic	Value
Total (N)	194
Age range (y)	18-88
Mean ± SEM	47.9 ± 1.18
Sex: female, n (%)	117 (60.3)
Any respiratory-related diagnosis, n (% total)	62 (31.9)
Controls,* n (% total)	132 (68)

	History of aspirin sensitivity, n (%)	
Diagnosis	**Present**	**Absent**
Asthma (n = 53)	16 (30.1)	37 (69.8)
Allergic rhinitis (n = 13)	1 (7.7)	12 (92.3)
CRSwNP (n = 27)	15 (55.5)	12 (44.4)
CRSsNP (n = 16)	0 (0)	16 (100)

*These are not "healthy" controls; however, disorders that may plausibly confound the analysis have been excluded (mast cells disorders, angioedema, urticaria).

LTE4 measurement

Urinary LTE4 quantification was performed on 24-hour urine specimens using the LC-MS method developed at Mayo Medical Laboratories. Values were reported as pg/mg Cr, with upper limit of normal being 104 pg/mg Cr.

Statistical analysis

LTE4 values were log-normalized, and nonparametric Wilcoxon/Kruskall-Wallis (rank sums) test was used for comparing groups. Central tendency was reported as median and interquartile range calculated using log-antilog functions. For correlations, Spearman index was calculated. To understand the effect of covariates such as age, sex, and aspirin status, whole model effects were constructed using standard least squares method with emphasis on effect leverage. Logistic fit of urinary LTE4 levels by diagnoses was used to generate receiver operator characteristic (ROC) curves and area under the curve (AUC). Two-sided *P* value of less than .05 was considered statistically significant. Statistical and graphic softwares used were JMP 10.0 (SAS, Cary, NC) and Microsoft Office 2010 (Redmond, WA), respectively.

RESULTS

Characteristics of subjects in the study

The characteristics of the patients included in the study are presented in Table I. The criteria for inclusion of controls are presented in Table E1 and objective data related to the respiratory diagnoses of asthma, allergic rhinitis, and CRS are presented in Table E2 in this article's Online Repository at www.jaci-inpractice.org. Of the 194 patients in the study, females comprised 60% of the study population. Sixty-two patients (31.9%) in the study carried a respiratory-related diagnosis that was assigned after clinical care. The proportion of diagnoses among the respiratory-related diagnosis group was as follows: asthma (n = 53 [85%]),

TABLE II. Influence of age and sex on 24-h urinary LTE4 excretion

	Overall	Controls	Any respiratory diagnosis
Age			
Correlation coefficient (*P* value)	0.11 (.09)	0.22 (.01)	−0.06 (.62)
Sex			
Female			
Urinary LTE4 (pg/mg Cr), median (IQR)	64 (44-95.9)	63 (43-87)	80.6 (47-431)
Male			
Urinary LTE4 (pg/mg Cr), median (IQR)	68 (46-139.9)	65 (43.4-117)	74.2 (46-193)
P value	.38	.43	.58

IQR, Interquartile range.

history of aspirin sensitivity (n = 17 [27%]), allergic rhinitis (n = 13 [21%]), chronic rhinosinusitis with nasal polyps (CRSwNP) (n = 27 [51%]), or chronic rhinosinusitis without nasal polyps (CRSsNP) (n = 16 [30%]). In addition, patients with clinically ordered LTE4 measurement but without the above diagnoses or mast cell mediator-related disorders were included as controls (n = 132) (see Table E1). None of the patients included in the study was taking a 5-lipoxygenase inhibitor. History of aspirin sensitivity was present approximately in a third of those with asthma (n = 16 [30%]) and approximately half of the patients with CRSwNP (n = 15 [55.5%]). Most patients with allergic rhinitis (n = 12 [92%]) and all patients with CRSsNP (n = 16 [100%]) had no history of aspirin sensitivity (Table I).

Age and sex effect on urinary LTE4

To ascertain the baseline influence of age and sex on LTE4 excretion, we analyzed the correlation of age and sex on overall study population, controls, and those with any respiratory-related diagnosis (Table II). There was a minor degree of correlation ($R = 0.2$) with age in controls ($P < .05$) but no significant correlation overall or in the respiratory group. There was lack of significant difference in 24-hour excretion of LTE4 between men and women in the overall analysis, controls, or those who had any respiratory diagnosis.

Urinary LTE4 levels distinguish CRS NP and history aspirin sensitivity

Next, we performed univariate analysis of LTE4 levels on each of those diagnoses (Table III). First, we compared the distribution of log transformed LTE4 values between respiratory diagnosis group versus controls to derive measures of central tendency (Figure E1). Then we compared univariately those patients with diagnosis of history of aspirin sensitivity versus all the remainder who did not have that diagnosis. Similar analysis was performed with diagnosis of asthma, allergic rhinitis, CRSwNP, and CRSsNP or any respiratory diagnosis. Subjects with aspirin sensitivity, asthma, CRSwNP, or any of the respiratory diagnoses had significantly higher values of LTE4 excretion ($P < .05$) than those without those respective diagnoses. Allergic rhinitis and CRSsNP were not associated with significant increases in urinary LTE4 excretion. Because aspirin sensitivity has been shown to be a determinant in driving urinary LTE4 excretion,[8,11,12,15] we performed additional analysis to adjust for the presence of history of aspirin sensitivity as a covariate. In this model, urinary LTE4 excretion was no longer significantly different between those who had asthma versus those who did not have the diagnosis of asthma ($P = .18$) or those patients with any respiratory diagnosis versus those without ($P = .73$). Urinary LTE4 levels in patients with a diagnosis of CRSwNP ($P = .01$) remained significantly elevated after adjustment for history of aspirin sensitivity. Of the 17 patients who had aspirin sensitivity in their clinical diagnosis, 11 had undergone an aspirin challenge of which 10 had provocation of symptoms (positive challenge). One patient had history of aspirin-induced wheezing but did not have any symptoms on challenge (urinary LTE4 level was 580 pg/mg Cr). Median (interquartile range) 24-hour urinary LTE4 level in aspirin-sensitive challenge-positive patients was 588 pg/mg Cr (375.3-1831.4pg/mg Cr) compared with 62 pg/mg Cr versus everyone else ($P < .001$).

Utility of 24-hour urinary LTE4 as a diagnostic test in discrimination of allergic rhinitis, asthma, CRSwNP, CRSsNP, and aspirin sensitivity

To ascertain the utility of urinary LTE4 as a diagnostic test, we performed ROC analysis for each of the conditions on their own in the study (Figure 1). The AUC was the highest for urinary LTE4 in discriminating history of aspirin sensitivity (AUC = 0.87), followed by CRSwNP (AUC = 0.78), asthma (AUC = 0.62), allergic rhinitis (AUC = 0.58), and finally CRSsNP (AUC=0.49). Because the best AUC in the ROC space was the ability of urinary LTE4 to discriminate those with history of aspirin sensitivity versus those without, we performed additional testing to ascertain diagnostic parameters at selected cutoff levels (we selected predefined values to study: 50, 80, and 951 pg/mg Cr) along with additional analysis for those who had a challenge-confirmed aspirin sensitivity. The normal values of LTE4 have been reported to be below the cutoff values for 50 and 80 pg/mg Cr previously.[8,16] In addition, the value of 951 pg/mg Cr was chosen because it was the closest value in our analysis to 859 pg/mg Cr, a suggested value with high specificity.[11] The specificity, sensitivity, positive predictive value (PPV), and negative predictive value (NPV) for these values for presence of history of aspirin sensitivity and for challenge-confirmed aspirin sensitivity are presented in Tables IV and V, respectively. The concentration at which 24-hour urinary LTE4 used as a test could discriminate history of aspirin sensitivity alone before a challenge with best balance of test parameters was 166 pg/mg Cr (sensitivity of 76.4% and specificity of 89.2%). The PPV and NPV at this cutoff were 40.6% and 97.5%, respectively, with a diagnostic odds ratio (DOR) of 27.02. If the analysis was performed on those confirmed to have a positive aspirin challenge and a history of aspirin sensitivity, the concentration at which 24-hour urinary LTE4 could discriminate this status with best balance of test parameters was 241 pg/mg Cr (sensitivity of 100% and specificity of 92%), with a PPV of 41.6% and an NPV of 100%.

DISCUSSION

LTE4 is a stable end product of the cystinyl leukotriene pathway and its measurement in urine as a biomarker of activity of this metabolic pathway is well validated. Increased LTE4 excretion has been demonstrated in allergen-induced asthma, asthma exacerbations, aspirin challenge, and increased basal excretion in AERD.[11-13,17-19] In this study, we wished to

TABLE III. Univariate analysis of urinary LTE4 excretion based on diagnoses

Diagnosis of	Yes	Vs everyone else	*P* value*	*P* value†
History of aspirin sensitivity	17; 469 (142.3-955.4)	177; 61 (42-98)	<.001	—
Asthma	53; 88 (47.9-362.8)	141; 63 (43.4-92.9)	.007	.18
Allergic rhinitis	13; 60 (18.3-163.0)	181; 67 (46-116.9)	.29	.29
CRSwNP	27; 241 (74-571)	167; 61 (42-92)	<.001	.01
CRSsNP	16; 63 (49.2-190.4)	178; 65 (43.7-118.2)	.98	.69
Any respiratory diagnosis	62; 77 (47-314.9)	132; 63 (43.2-93.4)	.02	.73
Challenge-confirmed aspirin sensitivity	10; 588 (375.3-1831.4)	184; 62 (43.2-99.5)	<.001	NA

IQR, Interquartile range; *NA*, not available/applicable.
Number in each group; median values to closest integer and IQR: N; median (IQR).
**P* value unadjusted (Wilcoxon/Kruskall-Wallis rank sums).
†Adjusted for history of aspirin sensitivity as covariate. Two-sided $P < .05$ considered significant.

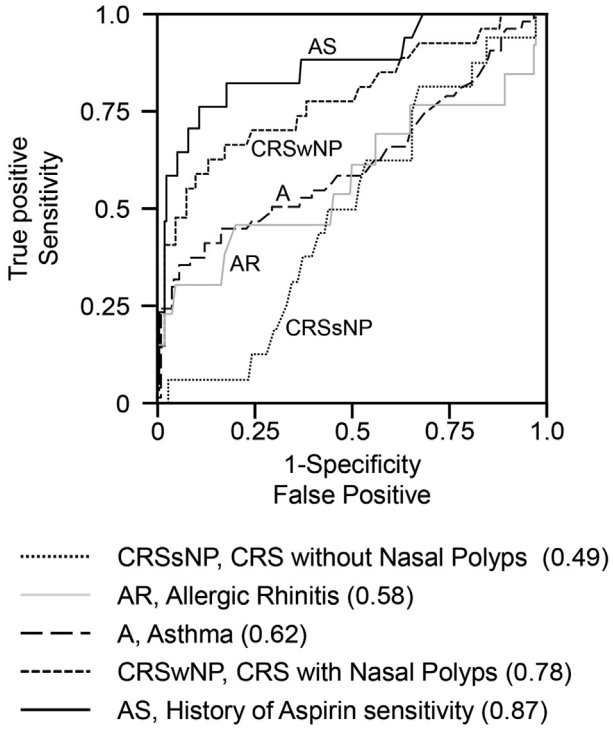

FIGURE 1. ROC curves for CRSsNP (dotted), allergic rhinitis (solid gray), asthma (wide dashes), CRSwNP (square dashes), and history of aspirin sensitivity (solid black). Numbers in parentheses represent the calculated AUC.

determine the diagnostic utility of 24-hour urinary LTE4 based on established and derived values. Because aspirin sensitivity strongly influences LTE4 excretion,[12,16] we adjusted for this status to account for the possible effect on analysis of CRSwNP and asthma states. In our study, LTE4 excretion after adjusting for aspirin sensitivity did not show a significant difference between those with asthma versus those without asthma. There may be a couple of reasons for this observation. Previous reports have demonstrated increases in LTE4 excretion in patients with asthma in acute exacerbation seen in emergency room visit for the flares.[19] Our study subjects were an elective outpatient population and not an emergency room cohort. In addition, the study may be underpowered and not be sufficient to ascertain whether asthma status independent of aspirin sensitivity was associated with significantly higher LTE4 excretion. Surprisingly, this was not the case for CRSwNP diagnoses, which met the threshold of significance even after adjusting for aspirin sensitivity status. This suggests that increased LTE4 excretion may be operating in CRSwNP even without aspirin sensitivity. However, this speculation needs to be verified by a prospective study involving aspirin challenge because the main risk of this study design could be that those subjects classified as aspirin tolerant did not have a confirmatory aspirin challenge; therefore, this population may include patients with "silent" AERD.

Established normal levels have been variably reported to be less than 50 pg/mg Cr[8] or a value between 67 and 80 pg/mg Cr based on method of assessment.[16,20,21] We chose these particular levels to test for specificity, sensitivity, PPV, NPV, and DOR in our study. For the diagnosis of history of aspirin sensitivity (Table IV) or for presence of challenge-confirmed aspirin sensitivity (Table V), these values are reported. In our analysis, a 24-hour urinary LTE4 level of 166 pg/mg Cr had the best balance of parameters, with sensitivity of 76%, specificity of 89%, PPV of 40%, and NPV of 97%, which suggested the presence of history of aspirin sensitivity. DOR is a single measure of test performance that combines sensitivity, specificity, and accuracy independent of prevalence.[22] DOR at a value of 166 pg/mg Cr was 27.02. For this condition, a value of 393 pg/mg Cr had the highest DOR of 61.7, with increased PPV (71.4%) but with reduced sensitivity (58.8%). Celejewska-Wojcik et al[11] have reported that for spot urinary LTE4 values ranging from 184 to 859 pg/mg Cr, specificity of discrimination increases from 37.5% to 93.7% between aspirin-tolerant patients with CRS and patients with AERD and have suggested a value of 859 pg/mg Cr as the best diagnostic value prechallenge. There may be a few reasons for this difference in observation. First, LTE4 values measured in 2 different laboratories or methods may not be directly comparable. The value in the previous study was derived from LTE4 measurements using immunoassay technique, whereas our observations are based on the commercially available LC-MS assay. This strongly suggests that values presented and interpretation of the cutoffs should be in the context of the method of analysis. There may be other values whose usefulness may lay in discriminating alternate situations; for example, Chiu et al[23] have recently reported that elevations of greater than 500 pg/mg Cr in urinary LTE4 levels were associated with high serum total IgE levels (≥100 kU/L) in children. Although, in our analysis, specificity of discrimination increased with higher values

TABLE IV. Specificity, sensitivity, PPV, and NPV at respective cutoffs for history of aspirin sensitivity before challenge

History of aspirin sensitivity	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Comments
50 pg/mg Cr	94.12 (71.3-99.8)	35.03 (28.0-42.5)	12.21 (7.1-19.0)	98.41 (91.4-99.9)	Normal values reported to be below this[8]
80 pg/mg Cr	88.24 (63.5-98.5)	62.71 (55.1-69.8)	18.52 (10.7-28.7)	98.23 (93.7-99.7)	Another upper limit of cutoff value reported for normal[16,21]
166 pg/mg Cr	76.47 (50.1-93.1)	89.27 (83.7-93.4)	40.62 (23.7-59.3)	97.53 (93.8-99.3)	Value in present analysis with best balance of diagnostic parameters
951 pg/mg Cr	23.53 (6.8-49.9)	98.87 (95.9-99.8)	66.67 (22.2-95.6)	93.09 (88.4-96.2)	Closest value in our study to 859 pg/mg Cr, another suggested value using immunoassay[11]

Numbers in parentheses represent 95% CI.

TABLE V. Specificity, sensitivity, PPV, and NPV at respective cutoffs for history of aspirin sensitivity and challenge-positive subjects

History of aspirin sensitivity and challenge positive	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Comments
50 pg/mg Cr	100 (69.1-100)	35.0 (28.0-42.5)	8.0 (3.9-14.2)	100 (94.2-100)	Normal values reported to be below this[8]
80 pg/mg Cr	100 (69.1-100)	62.7 (55.1-69.8)	13.1 (6.4-22.8)	100 (96.7-100)	Another upper limit of cutoff value reported for normal[16,21]
241 pg/mg Cr	100 (69.1-100)	92.0 (87.0-95.6)	41.6 (22.1-63.3)	100 (93.8-99.3)	Value in present analysis with best balance of diagnostic parameters
951 pg/mg Cr	40 (12.1-73.7)	98.8 (95.9-99.8)	66.6 (22.2-95.6)	96.6 (92.9-98.7)	Closest value in our study to 859 pg/mg Cr, another suggested value using immunoassay[11]

Numbers in parentheses represent 95% CI.

of urinary LTE4, the sensitivity declined at such higher values along with reduction in DOR. Micheletto et al[16] compared the urinary LTE4 excretion between normal controls, those with mild persistent atopic asthma, those with aspirin-induced asthma with rhinitis, or those with aspirin-induced asthma with polyps using immunoenzymatic assay.[16] In this study, patients with aspirin-intolerant asthma and NP demonstrated greatest excretion of urinary LTE4 (432.3 ± 88.1 pg/mg Cr) compared with those with aspirin-intolerant asthma with rhinitis without polyps, those with atopic asthma, or controls. Although this analyte can be measured by immunoenzymatic methods, mass spectroscopy has been recently recommended as the method of choice for measurement.[8,15] Because this study used an LC-MS method to assay urinary LTE4 levels, the data presented in our study are in alignment with such recommendations as well as clinically applicable.

There are several limitations to this study. First, this was a retrospective design and as a result is subject to the inherent limitations of such analysis. Patients with well-defined diagnoses were not specifically enrolled in the study; rather, inclusion in the analysis was based on history of LTE4 measurement. Second, this is the experience of a single tertiary care center; hence, the possibility of a referral bias exists. Third, though we excluded potential confounding conditions such as mast cell—related diseases,[24,25] those without assigned respiratory-related conditions designated as controls are not "healthy" or normal. Thus, the prevalence of the diagnosis being studied is based on this narrowly defined cohort and may not be applicable to general populations. A disadvantage in strict sense, it does, however, attempt to replicate the "mixed-bag" patient population seen in an allergy immunology practice. Fourth, because of the retrospective nature of the data and urine collections done outside of clinical area, we unfortunately do not have confirmation that the samples provided by the patients were indeed full 24-hour collections thought the test was ordered as such. Fifth, the analysis is on LTE4 measurements using the LC-MS method and may not be universally applicable to other methods of measuring LTE4 (eg, immunoassay). Last, aspirin challenge is not routinely performed in those patients who do not endorse lack of aspirin sensitivity to confirm absence of such proclivity. Thus, there may be patients with AERD inappropriately assigned to the other groups. Of those who had a history of aspirin sensitivity, 10 of 17 (59%) patients with aspirin sensitivity underwent a positive challenge to confirm the diagnosis.

Urinary LTE4 in aspirin-sensitive challenge-positive patients was significantly greater than in those in whom a challenge was not applicable. Because of the potentially life-threatening nature of a challenge, lack of availability in many centers, or clinical hurdles in performing challenges, clinical criteria have been recently suggested.[26] This analysis provides a cutoff value of 166 pg/mg Cr for discriminating history of aspirin sensitivity before a formal challenge and may inform the clinician whether the possibility of aspirin sensitivity exists. A higher cutoff of 241 pg/mg Cr was associated with challenge-positive aspirin sensitivity with 92% specificity. Thus, inclusion of additional noninvasive tests such as urinary LTE4 or measurement of proportion of platelet-adherent leukocytes[10] in peripheral circulation might further enhance the diagnostic ability.

This analysis provides important insights. The medical diagnoses used to define the respiratory-related condition or to assign controls were not patient volunteered diagnoses but were based on electronic medical records after clinical evaluation by the physician. Second, we used the values obtained from a clinically validated LC-MS method of detecting urinary LTE4. Third, we used a 24-hour sample for measuring the urinary LTE4. Although generally considered to be stable, significant variation in LTE4 may exist with asthma,[14] which, in turn, may affect the spot results. Greater coefficient of variability was noted

within day measurements in those with asthma compared with the normal group but no systematic periodicity (ie, diurnal variation) was identified.[14] A pooled 24-hour sample could potentially account for such variation; however, a large formal study comparing the value of a spot assay versus a 24-hour urine assay by the LC-MS method may clarify the degree of difference between both collection techniques. Fourth, although some of the individual respiratory diagnoses were infrequent (eg, allergic rhinitis) given the small sample size, it is plausible that the ROC estimates are not significantly different; however, this is a relatively large cohort that underwent 24-hour urinary LTE4 testing, providing an overview of the diagnostic utility of this testing with relatively high granularity. In summary, urinary LTE4 elevations may be seen in those with asthma and those with CRSwNP but influenced by underlying aspirin sensitivity. Elevation is significant in those with CRSwNP even after adjusting for diagnosis of history of aspirin sensitivity. ROC analysis of 24-hour urinary LTE4 suggests its value in discriminating aspirin sensitivity with lower utility in discriminating CRSwNP. Furthermore, our data indicate a cutoff value of 166 pg/mg Cr to suggest the presence of a history of aspirin sensitivity whereas a cutoff value of 241 pg/mg Cr discriminated challenge-confirmed aspirin-sensitive subjects with 92% specificity.

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