

Urinary Leukotriene E4 to Determine Aspirin Intolerance in Asthma: A Systematic Review and Meta-Analysis



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What is already known about this topic? Arachidonic acid pathway mediators such as leukotrienes are important in the pathogenesis of asthma. Urinary leukotriene E4 (ULTE4) increases following aspirin challenge. Increased basal ULTE4 is associated with the aspirin-intolerant asthma phenotype.

What does this article add to our knowledge? This systematic review and meta-analysis defines the diagnostic testing accuracy of ULTE4 in determining aspirin intolerance in asthma.

How does this study impact current management guidelines? This study demonstrates that a noninvasive urinary marker may be able to determine the risk of aspirin intolerance and thereby allow for precise categorization and management of this asthma phenotype.

BACKGROUND: Urinary leukotriene E4 (ULTE4) may be a biomarker that distinguishes aspirin-intolerant asthma from other asthma subtypes.

OBJECTIVE: To estimate the diagnostic testing accuracy of ULTE4 as a marker of aspirin intolerance in patients with asthma using previously published studies.

METHODS: We identified relevant clinical studies from a systematic review of English and non-English articles using MEDLINE, EMBASE, and CENTRAL (inception to February 10, 2015). Articles were screened at the abstract and full-text level by 2 independent reviewers. We included previously published studies that analyzed ULTE4 in human subjects with asthma characterized as having or not having aspirin intolerance on the basis of a specified definition: convincing history of aspirin intolerance, positive aspirin challenge, or both as the criterion standard. Individual-level data points from all included studies were obtained and analyzed.

RESULTS: The search strategy identified 867 potential articles, of which 86 were reviewed at the full-text level and 10 met criteria for inclusion. The sensitivity, specificity, positive predictive value, and negative predictive values of ULTE4 to determine aspirin intolerance in subjects with asthma were 0.55, 0.82, 0.75, and 0.66 (Amersham-enzyme immunoassay); 0.76, 0.77, 0.70, and 0.78 (Cayman-enzyme immunoassay); 0.70, 0.81, 0.86, and 0.79 (mass spectrometry); and 0.81, 0.79, 0.65, and 0.88 (radioimmunoassay) at optimal thresholds of 192, 510, 167 to 173, and 66 to 69 pg/mg Cr, respectively. The diagnostic odds ratio for each methodology was 6.0, 11.9, 10.5, and 19.1, respectively.

CONCLUSIONS: ULTE4 is a marker for aspirin-intolerant asthma and could potentially be used as a clinical test to identify the risk of aspirin intolerance in subjects with asthma. © 2016 American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2017;5:990-7)

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Conflicts of interest: J. B. Hagan is employed by Mayo Foundation and has received research support from the National Institutes of Health (NIH), GlaxoSmithKline, MedImmune, AstraZeneca, Teva, and Sanofi. T. M. Laidlaw has received research support from the NIH. R. Divekar is employed by Mayo Clinic and has an unrelated web blog through Google.com. H. Kita has received research support from the National Institute of Allergy and Infectious Diseases and the National Heart, Lung, and Blood Institute and has received lecture fees from Novartis. D. Lal has received research support from Mayo Clinic (intramural grants) and Flinn Foundation (award for microbiome research) and has received travel support from the University of Arizona. The rest of the authors declare that they have no relevant conflicts of interest.

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Abbreviations used

A-EIA- Amersham-enzyme immunoassay
AERD- Aspirin- exacerbated respiratory disease
AIA- Aspirin-intolerant asthma
ATA- Aspirin-tolerant asthma
C-EIA- Cayman-enzyme immunoassay
EIA- Enzyme immunoassay
MS- Mass spectrometry
NSAID- Nonsteroidal anti-inflammatory drug
RIA- Radioimmunoassay
ULTE4- Urinary leukotriene E4

An estimated 300 million persons in the world have asthma.¹ A history of aspirin intolerance has been reported in 7% of subjects with a diagnosis of asthma and in 15% of subjects with severe asthma.² Aspirin intolerance has been associated with asthma severity.³ Severe asthma has been associated with elevated urinary leukotriene E4 (ULTE4) levels.⁴ Increases in ULTE4 have been found during asthma exacerbations⁵ as well as during challenge with allergens⁶ and with nonsteroidal anti-inflammatory drugs (NSAIDs) such as aspirin.⁷ In contrast to corticosteroids, which are reported to have little effect on ULTE4, lipoxygenase inhibitors are known to reduce ULTE4 levels.⁸

The National Institute of Allergy and Infectious Diseases assembled a panel of experts to identify potential asthma outcome biomarkers. Among 10 potential biomarkers, ULTE4 was recognized along with total blood eosinophils, fractional exhaled nitric oxide (FENO), sputum eosinophils, and total and allergen specific IgE as a standardized supplementary biomarker intended to facilitate standardization of specific asthma outcomes in future clinical research studies of agents that may affect cysteinyl leukotriene activity. According to the expert group, ULTE4 measurements should be considered for studies that characterize asthma phenotypes, such as aspirin-intolerant, adult-onset, and eosinophilic asthma.⁸ The primary aim of this systematic review and meta-analysis was to evaluate the diagnostic testing accuracy of ULTE4 in determining aspirin intolerance in asthma.

METHODS

Eligibility criteria and literature search

This systematic review and meta-analysis contains the elements of the 27-item checklist put forth in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement.⁹ With the aid of an experienced medical librarian (P.J.E.), we performed a search to identify research or clinical studies analyzing urine for leukotriene E4 in human subjects with asthma wherein subjects were characterized as aspirin intolerant or not aspirin intolerant on the basis of a specified definition such as a convincing history of aspirin intolerance or an aspirin challenge or both. There were no age restrictions. We excluded studies when primary individual-level ULTE4 values were not available as data in the article, in supplementary material, on 2 or more requests from the primary author of the publication, or in figures with discrete subject-specific information whereby data could be derived in subjects with asthma with and without aspirin intolerance.

We performed a systematic review of English and non-English articles using MEDLINE, EMBASE, and CENTRAL (inception to February 10, 2015). Search terms formulated with the assistance

of a medical librarian experienced in systematic review methodology included leukotriene E4, urine or urinalysis or urinary, asthma or bronchospasm or nasal or nasal polyp and aspirin or acetylsalicyl or aspirin (ASA), aspirin-exacerbated respiratory disease (AERD). The detailed search strategy used for MEDLINE is listed in Table E1 in this article's Online Repository at www.jaci-inpractice.org. The MEDLINE search strategy was modified to accommodate the controlled vocabulary for EMBASE and CENTRAL. In addition, we reviewed the reference sections of eligible studies and searched articles that had been cited by the eligible articles using Thomson Reuters Web of Science. We contacted several content experts and inquired about their knowledge of any studies that were not on our list of eligible studies or of any unpublished data pertinent to our research question.

Study selection

Two reviewers working independently considered the potential eligibility of each of the abstracts that resulted from executing the search strategy. The reviewers were not blinded to the author, institution, or journal of publication. The reviewers determined whether the abstract met criteria to advance to full-text review, did not meet criteria, or whether this was not clear on the basis of the abstract review. Full-text articles were obtained unless both reviewers determined that the abstract was ineligible for this study. For articles that could not be obtained in full text through the institution's interlibrary loan process and where disagreement existed between 2 reviewers about advancing to the full-text review stage, a third reviewer assessed the abstract for criteria to advance to the full-text review stage. If 2 of the 3 reviewers recommended a full-text review, additional efforts were made to obtain the full-text articles. Then, 2 reviewers working independently considered each full-text report for eligibility. Disagreements about inclusion of full-text articles were harmonized by consensus; if this was not possible, arbitration by an additional content expert was arranged. One reviewer considered the potential eligibility of each of the abstracts that resulted from the reference sections of eligible studies and searched articles that had been cited by the eligible articles.

Data extraction

Data were extracted using a standardized form including descriptions of patients' demographic characteristics, methodologies of aspirin/NSAID challenge, methodologies used to analyze ULTE4, corresponding units of ULTE4 measurement, and country of origin of the study. Individual-level data points from all studies were determined either through obtaining original data points from authors or using an automated program to estimate data points from published figures.¹⁰ Analysis of published figures to assess the presence or absence of discrete data points and extraction of data were performed in duplicate by 2 reviewers. The results of analyses by duplicate reviewers were highly correlated (Spearman $\rho > 0.95$; $P < .0001$). Disagreements were resolved by consensus. Authors were contacted if data were missing.

Quality assessment

The risk of bias was assessed using the Cochrane Handbook for Systematic Reviews of Diagnostic Test Accuracy by 2 independent reviewers.¹¹ To assess the methodological quality of each diagnostic test accuracy study, we determined the spectrum of representative patients, the likelihood the reference standard (ULTE4) was used to classify the target condition (aspirin-intolerant asthma [AIA]), the time period between the reference standard and the target condition, the allocation of the reference standard on the study population with

regard to the whole group and independence on index test, independence of interpretation of tests, availability of clinical tests, interpretation of equivocal tests, and study withdrawals.¹¹ Disagreements about the risk of bias assessments were harmonized by consensus.

Data synthesis

Individual-level data points from all studies were determined either through obtaining original data points from authors or using an automated program to estimate data points from published figures.¹⁰ For each laboratory methodology, the generalized estimating equation model was used to estimate the sensitivity and specificity for each potential cutoff point of ULTE4 by specifying an exchangeable working correlation structure for patients within the same study.¹² Receiver-operating characteristic curves were then constructed by plotting the estimated sensitivity and 1 minus specificity using a y by x scatter plot. Area under the curve was calculated by using natural spline interpolation. Youden index was used as the criteria to choose the optimal cutoff point for each receiver-operating characteristic curve. Positive predictive value, negative predictive value, diagnostic odds ratio, and its 95% confidence interval were estimated for the chosen optimal cutoff point for each laboratory method using the generalized estimating equation method adjusting for within-study correlation. The diagnostic odds ratio is a single indicator of diagnostic test performance where better test performance is represented by higher values ranging from 0 to infinity. The diagnostic odds ratio represents “the ratio of the odds of positivity in disease relative to the odds of positivity in the nondiseased.”¹³ Summary statistics of nonparametric data were expressed as the median, range, and interquartile ranges, with significance between groups determined by the Wilcoxon test. P values of less than .05 were considered statistically significant. JMP 10.0.0 (SAS Institute Inc., Cary, NC) and SAS Studio 9.3 (SAS Institute Inc.) software were used to conduct the analyses.

Subgroup analyses

To explore sources of heterogeneity, we compared ULTE4 values from patients with disease using the categories AERD, AIA, aspirin-tolerant asthma (ATA), and nonasthma (normal) controls. Included studies were published between 1991 and 2015 during which the terminology of AERD was introduced around 2002. To attempt to carefully interpret the previously published literature and in recognition of the inherent lack of optimal documentation regarding the absence or presence of nasal polyps and/or rhinitis or rhinosinusitis in the literature preceding 2002,¹⁴ the categories AERD and AIA were analyzed separately. Thus, for the purpose of this study, AIA was defined by asthma diagnosis and aspirin intolerance but without author-defined AERD or clinical criteria such as nasal polyps included in the study to characterize AERD in an individual study subject. In contrast, for the purpose of this study, AERD was defined as AERD specifically stated by the author in the publication or when the combination of asthma, nasal polyps, and aspirin intolerance was clearly identified in a specific study subject within the publication or in information obtained from the study author. The distinctions required to optimally interpret the previous literature were made with the understanding that these conditions may be regarded at this time as a continuum of the same group and that subjects previously categorized with AIA could have AERD if further evaluated for nasal polyps and rhinosinusitis.

RESULTS

Our initial search identified 398 articles. Additional strategies of searching bibliographies, reviewing lists of works cited, searching clinical trial registries, and contacting experts identified 642 unique citations. After removal of duplicates, there were 867 citations from which 86 met criteria for full-text review. Nine articles could not be obtained in full text through our institution's usual interlibrary loan process. Ten articles met all criteria and were included in qualitative synthesis (Figure 1). There were no unpublished data identified that fulfilled the inclusion criteria. The characteristics of the included studies are summarized in Table I.^{7,15-23} All the studies included adults. The number of participants in each study arm ranged from 6 to 83. The studies were performed in the United States ($n = 2$), Europe ($n = 5$), and Japan ($n = 3$). Methodologies of ULTE4 analysis included enzyme immunoassay (EIA) from 2 predominant sources (Amersham-EIA [A-EIA] and Cayman-EIA [C-EIA]), mass spectrometry (MS), and radioimmunoassay (RIA). Units of ULTE4 described in the article were in pg/mg cr ($n = 8$), pmol/mg cr ($n = 1$), and ng/mg cr ($n = 1$). pmol/mg cr and ng/mg cr were converted to pg/mg cr for this report. Funding sources were mixed between government ($n = 7$), university ($n = 1$), allergy/immunology society ($n = 1$), and private funding ($n = 2$).

Diagnosis of asthma differed across the included studies (see Table II) and ranged from implied and physician diagnosis to that consistent with American Thoracic Society or Global Initiative for Asthma guidelines or documented by bronchial hyperresponsiveness. All studies that measured lung function included patients with a mean FEV₁ percent greater than 70. The definition of aspirin challenge included oral aspirin as well as lysine aspirin administered intravenously. All but 2 of the included studies evaluated aspirin-intolerant patients who underwent or previously underwent witnessed aspirin challenges. Of the 2 studies that did not require witnessed challenges in all subjects, there were rigorous definitions of aspirin intolerance. One study included subjects with either witnessed challenge or “at least two histories of attack induced by NSAIDs.”¹⁷ The other study included subjects with witnessed challenge or an “obvious history of asthma attack precipitated by NSAID administration.”¹⁸ In contrast to older published reports, more recent studies clarified concomitant diagnoses with chronic rhinosinusitis, nasal polyps, and AERD.

Quality assessment

Results from the risk of bias assessment for each study are displayed in Table E2 in this article's Online Repository at www.jaci-inpractice.org. In general, we did not identify significant bias risks in the selected studies using this tool. The study populations appeared to represent predominantly adult subjects who presented to an academic medical center.

Overall analyses

ULTE4 varied widely in healthy study subjects ranging from a minimum of 2 by RIA to a maximum of 489 pg/mg cr in a healthy subject sample analyzed by EIA. Because of this variation as well as that attributed to methods of analysis of ULTE4 in previously published studies,²¹ results of ULTE4 in normal subjects who underwent analysis by 2 individual EIAs, MS, and RIA were compared. Although normal subjects' ULTE4 values performed by RIA and MS were not statistically different, significant differences were found when comparing ULTE4 levels

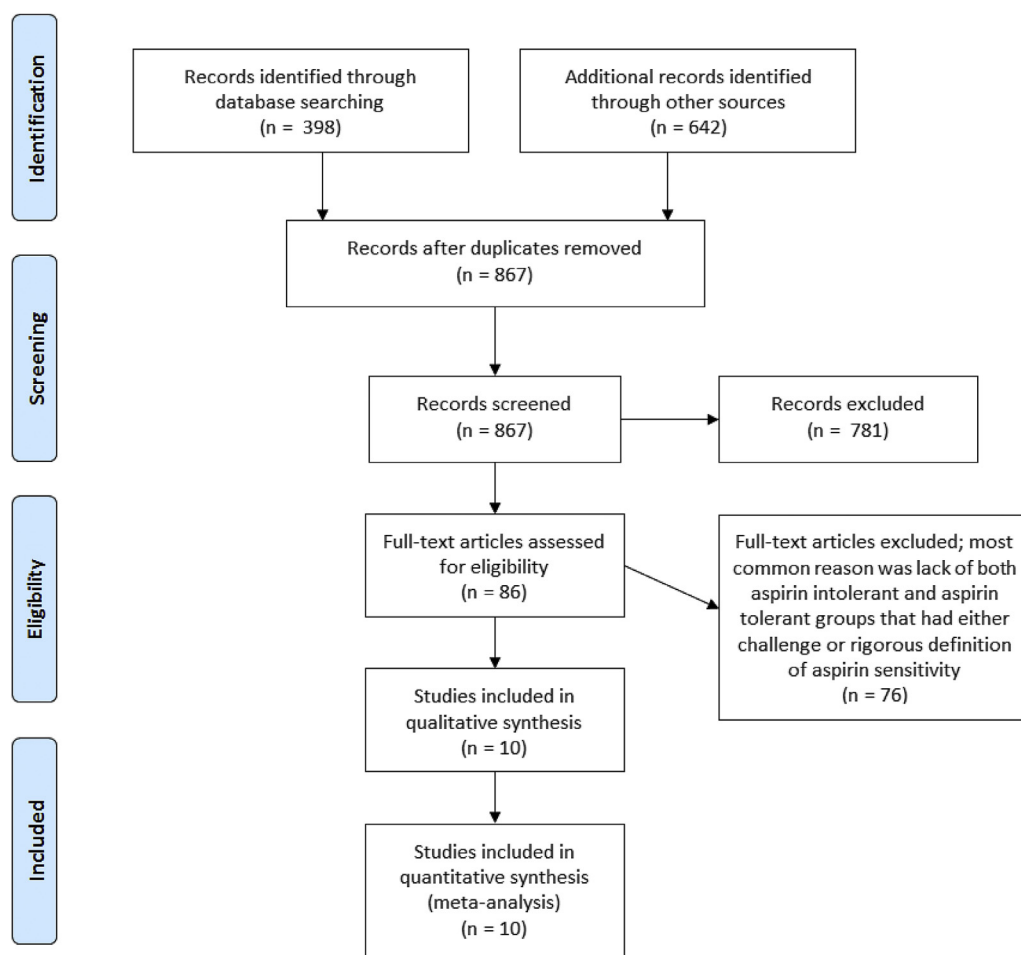


FIGURE 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses flow diagram describing the steps of study selection.⁹

TABLE I. Characteristics of included studies

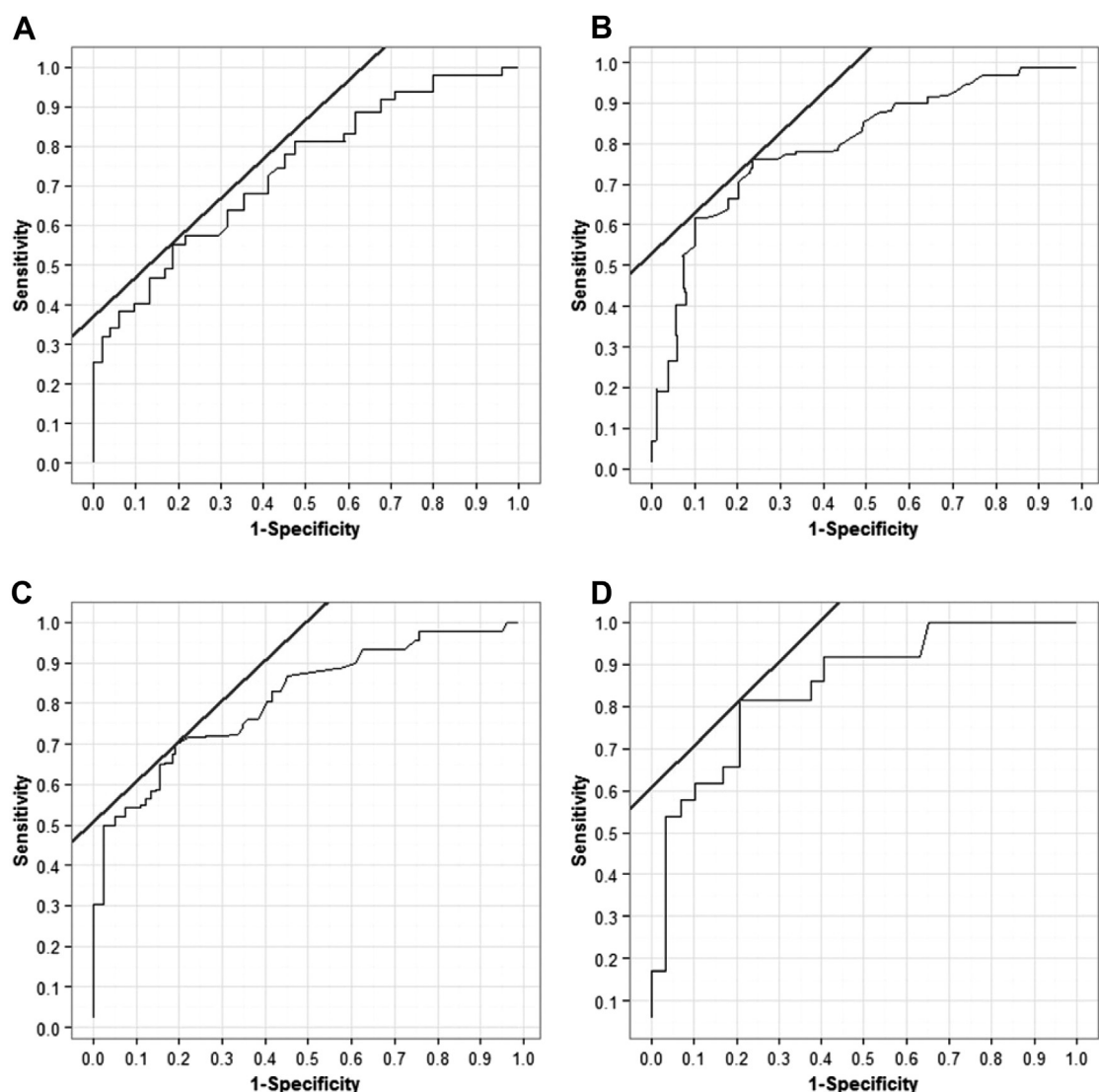
Author	AIA study no.	ATA study no.	Controls	Setting	Countries of origin	Mean ages (y)	Method of ULTE4 analysis	Original units of ULTE4	Funding
Cahill et al, ¹⁵ 2015	21	8		Academic	USA	>35	MS	pmol/mg cr	USA and private
Swierczynska-Krepa et al, ²³ 2014	20	14		Academic	Poland	>45	C-EIA	pg/mg Cr	University grant
Laidlaw et al, ¹⁹ 2012	10	9	8	Academic	USA	>35	MS	ng/mg cr	USA, AAAAI, private
Celejewska-Wojcik et al, ¹⁶ 2012	8	8		Academic	Poland	>40	C-EIA	pg/mg Cr	Switzerland grant
Ono et al, ²⁰ 2011	15	11		Academic	Japan	>51	C-EIA	pg/mg Cr	Not described
Sanak et al, ²¹ 2010	46/41	80/83	35/50	Academic	Poland	>30	MS/C-EIA	pg/mg Cr	Iceland, Liechtenstein, Norway, Poland, GA2LEN
Kawagishi et al, ¹⁸ 2002	47	51	33	Academic	Japan	>50	A-EIA	pg/mg Cr	Not described
Higashi et al, ¹⁷ 2002	13	9		Academic	Japan	>50	A-EIA	pg/mg Cr	Not described
Smith et al, ²² 1992	10	29	17	Academic	UK	>20	RIA	pg/mg Cr	UK
Christie et al, ⁷ 1991	6	6		Academic	UK	>20	RIA	pg/mg Cr	UK

AAAAI, American Academy of Allergy, Asthma & Immunology; GA2LEN, Global Allergy and Asthma European Network.

among each of the other modalities (Figure 2). Thus, analysis of the diagnostic testing accuracy of ULTE4 was determined for each methodology used to assess ULTE4 in the included studies.

A summary of ULTE4 values in subjects with ATA, AIA, and AERD is presented in Figure 2 along with normative control data

from the included studies. In general, values of ULTE4 were elevated in subjects with asthma when compared with normal subjects. ULTE4 levels in subjects with asthma with aspirin intolerance were elevated compared with subjects with asthma without aspirin intolerance. The phenotype AERD could not be



	AUC	Threshold	Sensitivity	Specificity	PPV	NPV	DOR [95% CI]
a. EIA Amersham	0.74	192	0.55	0.82	0.75	0.66	6.0 [4.0, 9.0]
b. EIA Cayman	0.78	510	0.76	0.77	0.7	0.78	11.9 [8.8, 16.0]
c. MS	0.8	167 to 173*	0.7	0.81	0.86	0.79	10.5 [4.5, 24.2]
d. RIA	0.84	66 to 69*	0.81	0.79	0.65	0.88	19.1 [4.0, 92.4]

* Cutoff points yielding equivalent sensitivity and specificity

FIGURE 3. Summary of receiver-operating curves for determining aspirin intolerance by ULTE4.

DISCUSSION

ULTE4 is an important marker of cysteinyl leukotriene activity in patients with asthma.⁸ Inhalation of leukotriene E4 results in a time-dependent increase in histamine responsiveness with maximal effect in 7 hours and lasting up to a week.²⁴ After ingestion of aspirin, eosinophil progenitor cells are released by the bone marrow and histamine and leukotrienes are released from the upper and lower respiratory mucosa in patients who experience a systemic reaction.^{25,26} After bronchial challenge with nebulized leukotriene D4, Verhagen et al²⁷ identified a significant rise in ULTE4 over 12 hours with 50% of the total

amount occurring within 2 hours of the challenge. From this he proposed that what was previously described as sulfidopeptide leukotriene formation in the lungs could be monitored by ULTE4.²⁷ To date a number of studies have been performed to evaluate ULTE4 in subjects with asthma. This is the first systematic review and meta-analysis to our knowledge that has examined the diagnostic testing accuracy of ULTE4 in determining aspirin intolerance in subjects with asthma. Because measurements of ULTE4 by immunoassay and HPLC-MS have been previously shown to differ,²¹ ULTE4 levels in this study were evaluated on the basis of the methodology used for analysis

in each previously published study. The selected studies that comprised analyses by 2 specific immunoassays, MS and RIA, all showed significant increases in ULTE4 in subjects with asthma who were intolerant to aspirin compared with subjects with asthma who tolerated aspirin. An optimal threshold was established and the diagnostic testing accuracy of ULTE4 was determined for each individual methodology. The sensitivity, specificity, positive predictive value, and negative predictive values of ULTE4 to determine aspirin intolerance in subjects with asthma were 0.55, 0.82, 0.75, and 0.66 (A-EIA); 0.76, 0.77, 0.70, and 0.78 (C-EIA); 0.70, 0.81, 0.86, and 0.79 (MS); and 0.81, 0.79, 0.65, and 0.88 (RIA) at optimal thresholds of 192, 510, 167 to 173, and 66 to 69 pg/mg Cr, respectively. The diagnostic odds ratio for each methodology was 6.0, 11.9, 10.5, and 19.1, respectively.

NSAIDs including aspirin are important medications that may result in clinically significant exacerbations of asthma.^{28,29} A recent meta-analysis found aspirin intolerance present in 7% of subjects with a diagnosis of asthma and in 14.89% of subjects with severe asthma.² Aspirin intolerance has been associated with severe and near-fatal asthma.^{30,31} However, the risk of fatality attributed to aspirin intolerance may be mitigated by avoiding aspirin and other NSAIDs.³¹ To date there is no generally recognized, clinically available test to determine the presence of aspirin intolerance. The specificity of a medical history of aspirin intolerance was found in one study to be 96% but the sensitivity in the same study was reported to be only 52%.³² A recent retrospective analysis of 24-hour ULTE4 was 89% sensitive in determining a history of aspirin sensitivity in subjects with allergic rhinitis, asthma, and chronic rhinosinusitis using MS and an optimal cutoff value of 166 pg/mg Cr.³³ At present, aspirin challenge serves as the criterion standard for diagnosis.^{4,34,35} Recognizing the presence of aspirin intolerance may have important implications regarding disease severity, need for medication avoidance, risk of concomitant nasal or sinus disease that may contribute to asthma, and therapeutic options including aspirin desensitization in patients with AERD, and could perhaps provide mechanistic insight into the pathogenesis of asthma.^{4,34-37}

This study is also the first systematic review and meta-analysis to our knowledge to provide a summary of normative values of ULTE4 levels stratified according to the major methods of analysis used to analyze ULTE4. These normative values may serve as a major step to advance the standardization of measurements of ULTE4 in humans. These values should serve as a reference for interpretation of the previous literature identifying ULTE4 measurements in humans. This compilation of normative data may be used as preliminary data for individual clinical laboratories, research investigators, or pharmaceutical companies while planning optimal assessment of cysteinyl leukotrienes in future clinical research trials as has been recommended by the National Institute of Allergy and Infectious Diseases expert panel.⁸ There are patients with either severe or nasal polyp-associated asthma who are currently avoiding aspirin and thus have no clinical history to offer as a clue to the presence or absence of aspirin intolerance. This noninvasive test may help identify the potential risk of aspirin intolerance in these patients.

There are several limitations to this systematic review and meta-analysis. First, studies of ULTE4 in asthma were performed over a range of 25 years using different methodologies of assessing ULTE4. To account for these differences, we compared

ULTE4 levels of subjects with ATA and AIA within groups according to the methodology used for analysis. Second, we were not able to ascertain individual-level data from authors of a number of the previously published studies. For those studies in whom primary study data were unavailable but in which discrete information was presented in figures in the final publications, we were able to obtain subject-specific ULTE4 levels. However, we were unable to obtain subject-specific demographic data to compare age- and sex-based differences in ULTE4 in these populations. Studies were excluded if primary individual-level data were not available and if figures with discrete subject-specific information were not presented in figures. Thus, the present study was limited because a significant number of publications on the topic by well-respected authors did not meet our search criteria. Third, most of the studies were conducted at academic institutions and study populations where referral bias may suggest a lack of generalizability of these findings to patients undergoing evaluation at community-based clinics. Fourth, information in the studies that met inclusion criteria was insufficient to draw a formal conclusion regarding the potential relationship between the anatomical volume of sinus mucosal thickening or polyps and ULTE4 in this meta-analysis. Fifth, recent publications have been more rigorous in documenting the presence or absence of nasal polyps, chronic rhinosinusitis, and AERD. Absence of these categorizations in the older studies did not allow separation of the patients with AERD from those diagnosed with AIA at the time. Because of this limitation, we recommend that future studies provide individual patient data regarding the presence or absence of nasal polyps, chronic rhinosinusitis, and AERD diagnosis. Finally, all included studies that by definition required a rigorous definition of aspirin intolerance and frequently observed aspirin challenge included adults. Thus, these results may not directly apply to children with asthma or young adults in whom the possibility of aspirin intolerance may be considered.

In summary, the results of this systematic review and meta-analysis have defined the diagnostic testing accuracy of ULTE4 in determining aspirin intolerance in asthma. Our findings have confirmed the need to define methodology of analysis when comparing ULTE4 levels in subjects with asthma. This study has provided preliminary data regarding normative values for each of the primary methodologies for analyzing ULTE4 in human subjects. Additional research is needed to standardize age- and sex-based normative values and define the clinical utility of this test in prospective studies as a diagnostic biomarker for specific subtypes of asthma.

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TABLE E1. Database search terms

No.	Search terms	No. of articles
1	Leukotriene E4/	898
2	Leukotriene E4/ur	272
3	ulte4.tw.	15
4	lte4.tw.	1,007
5	(leukotriene adj (e4 or “e 4”)).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]	1,078
6	1 or 3 or 4 or 5	1,578
7	6 and (ur.fs. or urine.mp. or urinary.mp.) [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]	501
8	2 or 7	501
9	exp Asthma/or Nasal Polyps/or samplers triad.mp.	1,11,429
10	8 and (asa or aspirin* or aerd or acetylsalicyl*).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]	89
11	8 and (asthma* or polyp*).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]	274
12	8 and 9	237
13	10 or 11 or 12	285

TABLE E2. Quality assessment of diagnostic accuracy of included studies

Methodological quality summary	Study no.									
	1	2	3	4	5	6	7	8	9	10
Was the spectrum of patients representative of those who will receive the test in practice?	U	U	U	No	U	U	U	U	U	U
Is the reference standard likely to classify the target condition correctly?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Is the time period between reference standard and index tests short enough that condition did not change between the 2 tests?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Did the whole sample or a random selection of the sample receive verification using the intended reference standard?	No	Yes	No	Yes	Yes	U	No	No	No	U
Did patients receive same reference standard regardless of index test result?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Was reference standard independent of the index test?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Were index and reference standard tests interpreted independently?	U	U	U	U	U	U	U	U	U	U
Were the same clinical data available when interpreting tests that would be available in clinical practice?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Were uninterpretable or intermediate results reported?	U	U	U	Yes	U	U	U	U	U	U
Were withdrawals from the study explained?	U	Yes	U	Yes	U	U	No	U	U	U

U, Unclear.

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