

Diagnostic Accuracy of Urinary LTE₄ Measurement to Predict Aspirin-Exacerbated Respiratory Disease in Patients with Asthma



Grazyna Bochenek, MD, PhD^a, Tomasz Stachura, MD^a, Krystyna Szafraniec, PhD^b, Hanna Plutecka, PhD^a, Marek Sanak, MD, PhD^a, Ewa Nizankowska-Mogilnicka, MD, PhD^a, and Krzysztof Sladek, MD, PhD^a Krakow, Poland

What is already known about this topic? Patients with aspirin-exacerbated respiratory disease (AERD) are distinguished from patients with aspirin-tolerant asthma (ATA) by significantly higher baseline concentrations of urinary leukotriene E₄ (uLTE₄). However, an overlap between the individual values of the groups exists.

What does this article add to our knowledge? The uLTE₄ concentration equaling 800.0 pg/mg creatinine, as estimated by enzyme-linked immune assay using a spot morning urine sample, best discriminates patients with AERD from patients with ATA. However, uLTE₄ measurement by itself is not sufficiently useful for AERD diagnosis.

How does this study impact current management guidelines? A set of clinical parameters has a superior accuracy in the prediction of AERD diagnosis to the measurement of uLTE₄ concentration alone. The addition of uLTE₄ concentration to clinical parameters slightly enhances the prediction of AERD diagnosis, especially due a high negative predictive value.

BACKGROUND: Patients with aspirin-exacerbated respiratory disease (AERD) are distinguished from patients with aspirin-tolerant asthma (ATA) by significantly higher baseline concentrations of urinary leukotriene E₄ (uLTE₄). However, an overlap between the individual values of the groups exists.

OBJECTIVE: The objective of this study was to estimate the discriminative value of uLTE₄ concentration in differentiating between patients with AERD and patients with ATA and evaluate the diagnostic accuracy of uLTE₄ measurement alone and added to clinical parameters to predict AERD diagnosis in patients with asthma.

METHODS: Clinical data were collected from questionnaires. Spirometry, skin prick tests, total IgE, and blood eosinophilia were evaluated. uLTE₄ concentrations were measured in morning urine samples by enzyme-linked immune assay (ELISA).

RESULTS: Patients with AERD (n = 247) had significantly higher uLTE₄ concentrations than those with ATA (n = 239). The uLTE₄ concentration of 800.0 pg/mg creatinine as measured by ELISA on a spot sample best discriminated the 2 groups (area under the curve 0.7; 95% confidence interval 0.66-0.74, sensitivity 49%, specificity 81%). The positive predictive value and negative predictive value (NPV), after considering the prevalence of AERD in the population of asthmatics, were 16% and 96%, respectively. Nasal polyps, upper airway symptoms, nasal corticosteroid treatment, asthma exacerbations, forced expiratory volume in the 1 second predicted, and age of asthma onset were independent predictors of AERD diagnosis. The addition of elevated uLTE₄ concentration to the set of clinical parameters enhanced slightly the prediction of AERD diagnosis beyond the level predicted by clinical parameters (*P* = .036). **CONCLUSIONS:** A set of typical clinical parameters has a superior accuracy in prediction of AERD diagnosis than the measurement of uLTE₄ concentration alone. The addition of uLTE₄ concentration to clinical parameters slightly enhances the prediction of AERD diagnosis, especially due to a high NPV. © 2017 American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2018;6:528-35)

Key words: Aspirin; Aspirin-exacerbated respiratory disease; AERD; Aspirin-tolerant asthma; Leukotriene E₄; LTE₄; Urinary

^aDepartment of Internal Medicine, Faculty of Medicine, Jagiellonian University Medical College, Krakow, Poland

^bDepartment of Epidemiology and Population Studies, Institute of Public Health, Faculty of Health Sciences, Jagiellonian University Medical College, Krakow, Poland

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Corresponding author: Grazyna Bochenek, MD, PhD, Department of Internal Medicine, Faculty of Medicine, Jagiellonian University Medical College, Skawinska 8, 31-066 Krakow, Poland. E-mail: mmbochen@cyf-kr.edu.pl.

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Abbreviations used

AERD- Aspirin-exacerbated respiratory disease
ATA- Aspirin-tolerant asthma
AUC- Area under the curve
CI- Confidence interval
COX-1- Cyclooxygenase 1
CysLT- Cysteinyl leukotriene
ELISA- Enzyme-linked immune assay
FEV₁- Forced expiratory volume in the 1 second
FVC- Forced vital capacity
HC- Healthy control
LC-MS- Liquid chromatography-mass spectrometry
LTE₄- Leukotriene E₄
NPV- Negative predictive value
NSAID- Nonsteroidal anti-inflammatory drug
PPV- Positive predictive value
ROC- Receiver operating characteristic
TENOR- The Epidemiology and Natural History of Asthma:
Outcomes and Treatment Regimens
uLTE₄- Urinary leukotriene E₄

higher doses of corticosteroids to control the disease, as well as by particularly aggressive polypoid hypertrophy of sinus mucosa.^{2,3} The disease is associated with eosinophilic inflammation of the upper and lower airways.^{4,5} A central feature of AERD is a constitutive overproduction of bronchoconstrictive cysteinyl leukotrienes (CysLTs) attributed to the dysregulated function of the 5-lipoxygenase and leukotriene C₄ synthase pathways.⁵ Measurement of leukotriene E₄ (LTE₄), the stable end metabolite of CysLTs in urine, is recognized as a good marker of their systemic production.⁶ Baseline urinary LTE₄ (uLTE₄) concentrations in patients with AERD have been shown to exceed those from patients with ATA, which result in significantly higher mean or median values of this metabolite in the former group.⁷⁻¹³ However, there was a considerable overlap between the individual values of the 2 groups. In addition, the prior studies were carried out on relatively small groups of patients, not exceeding several dozen.

Diagnosis of AERD is based on typical clinical presentation and a history of asthma attacks and/or nasal reactions after ingestion of COX-1 inhibitors, although reliance exclusively on history is not advisable due to the risk of misdiagnosis.^{1,14} Aspirin challenge is the most reliable method of AERD diagnosis.¹⁵ However, the procedure is time consuming, must be supervised by an experienced medical staff due to the risk of adverse reactions, and is unsuitable for patients with severe asthma whose forced expiratory volume in the 1st second (FEV₁) is lower than 70% of the predicted value. Therefore, noninvasive methods that could assist in diagnosis are needed. Currently, none of the proposed *in vitro* tests can be recommended for this purpose.¹⁶

In this study, we aimed to estimate the discriminative value of uLTE₄ concentration measured by validated enzyme-linked immune assay (ELISA) in differentiating between patients with AERD and patients with ATA based on a larger sample size than in previous studies. We also evaluated the diagnostic accuracy of uLTE₄ measurement as both a single parameter and added to clinical parameters to predict AERD diagnosis in patients with asthma.

METHODS

Subjects

Three groups of subjects were studied. The first group comprised 247 patients with AERD recruited from consecutive patients diagnosed with AERD at the Department of Internal Medicine, Jagiellonian University Medical College, Krakow, Poland. The recruitment phase lasted from June 2008 to September 2013. In the majority of them, the diagnosis was based on a typical history confirmed with a positive aspirin challenge. In 42 (17%) severe steroid-dependent asthmatics, the diagnosis was based exclusively on an unequivocal clinical presentation and a history of asthma attacks after ingestion of nonsteroidal anti-inflammatory drugs (NSAIDs). They never qualified for aspirin challenge due to low FEV₁ and the nonfeasibility of reducing oral corticosteroid dose before the challenge. The second group comprised 239 patients with ATA. Each of them had occasionally used aspirin without any adverse effects. In 28 of them, selected at random, an aspirin challenge was performed with negative result in all cases. All patients were clinically stable, without any asthma exacerbation within the 6 weeks preceding the study. The healthy control (HC) group consisted of 95 nonatopic subjects with no history of asthma, allergy, or adverse reactions to NSAIDs.

The study protocol was approved by the Jagiellonian University Ethics Review Committee. Written informed consent was obtained from each participant.

Data collection

A specifically structured questionnaire was used to collect demographic and medical history data of patients with asthma. Asthma severity was estimated from the level of treatment required to control symptoms.¹⁷ Asthma treatment was divided into the following categories: (1) no corticosteroids; (2) inhaled corticosteroids at a daily dosage of ≤500 µg of fluticasone equivalent; (3) inhaled corticosteroids at a daily dosage of >500 µg of fluticasone equivalent; and (4) oral corticosteroids with inhaled corticosteroids.

Spirometry (MasterScreen, Jaeger, Wurzburg, Germany) was carried out at baseline and after administration of 4 salbutamol puffs (400 µg).

Skin prick tests were performed with 12 common aeroallergens (Allergopharma, Reinbek, Germany). Patients were deemed atopic when at least one positive test response was obtained.

The total IgE concentration was measured with the nephelometric method using NLatex IgE mono assembly kits (Dade Behring, Newark, NJ). Blood eosinophil counts were calculated in a Fuchs-Rosenthal chamber.

Urine collection

Morning urine samples were collected after a 2-hour accumulation of urine in the bladder. LTE₄ concentrations were measured in unpurified samples by ELISA (Cayman Chemical, Ann Arbor, Mich). The results were recalculated over urinary creatinine concentration and expressed in picograms per milligram of creatinine.¹⁸

Statistical methods

The primary analysis compared the AERD, ATA, and HC groups by standard procedures: the χ^2 test for categorical variables, and the Student *t*-test, Mann-Whitney *U* test, and Kruskal-Wallis test for continuous variables. The ability of LTE₄ concentration to discriminate between patients with AERD and patients with ATA was assessed in 2 ways. First, based on LTE₄ distribution in the HC group, the frequency distribution of patients with AERD and

patients with ATA in quartile groups was tested by the χ^2 test. As no differences were observed in the proportions of patients with AERD and patients with ATA in the second and third quartile, those intervals were joined. Secondly, the receiver operating characteristic (ROC) analysis was applied; sensitivity, specificity, and positive (PPV) and negative predictive values (NPV) were determined. The cutoff point giving the best Youden index and the area under the ROC curve (AUC) were calculated.

Logistic regression was used to model the relationship between clinical and laboratory parameters and diagnosis of AERD. A set of meaningful predictors among those both statistically and clinically significantly related to outcome in univariate analysis was determined by the backward elimination procedure. To study the additional effect of the increased LTE_4 concentration over parameters selected by the regression model, which are usually considered in clinical practice (model A), a dichotomized LTE_4 variable with a cutoff point determined by ROC analysis was added (model B). To determine whether model B enhanced the prediction of AERD diagnosis against model A, scores for models A and B were calculated on the basis of logistic regression coefficients (multiplied by 10), and then the scores were compared by the ROC analysis resulting in calculation of the difference between AUCs. To test the difference between scores A and B in the AERD and ATA groups, the Wilcoxon signed rank sum test was applied. All statistical calculations were performed with SPSS software version 22, except the ROC analysis, which was performed with MedCalc Statistical Software version 16.8.4 (MedCalc Software bvba, Ostend, Belgium; <https://www.medcalc.org>; 2016). A P value $<.05$ was considered statistically significant.

RESULTS

Clinical characteristics of the studied groups

Both groups of patients with asthma were comparable with respect to female predominance, age, body mass index, proportion of patients with positive skin prick test responses, and total IgE concentration (Table I). Patients with AERD had a longer duration and a more severe course of asthma, which was expressed both by corticosteroid use and the presence of exacerbations. A higher proportion of patients with AERD were treated with oral corticosteroids (26.6% vs 17.6%, $P = .02$), and a lower proportion were treated with inhaled corticosteroids at a daily dosage of ≤ 500 μg (32.4% vs 44.4%, $P = .007$) compared with the ATA group. Patients with AERD had more asthma exacerbations requiring medical interventions, and intensive care unit stays. They had more severe airway obstruction estimated by $\text{FEV}_1\%$ predicted and $\text{FEV}_1/\text{forced vital capacity (FVC)}$ ratio, both before and after bronchodilator use. A higher proportion of patients with AERD reported upper airway symptoms, nasal polyps, history of polypectomies, and nasal corticosteroid use. Blood eosinophil counts were higher in patients with AERD than in patients with ATA. The HC group had similar age, but the proportion of females was somewhat lower than in asthma groups. All HC subjects had negative skin prick test responses. The total IgE concentration and blood eosinophil counts were lower in HC subjects than in asthma groups.

Urinary LTE_4 concentrations in the studied groups and their applicability in AERD diagnosis

Patients with AERD had significantly higher uLTE_4 concentrations (median = 783.0 [Q1, Q3: 376.0, 2085.0] pg/mg creatinine) than patients with ATA (median = 408.0 [Q1, Q3:

207.0, 697.0] pg/mg creatinine) and HC (median = 493.0 [Q1, Q3: 341.0, 780.0] pg/mg creatinine) (Figure 1). No differences were found between patients with ATA and HC.

Based on the quartile distribution of uLTE_4 concentrations in HC subjects, it was demonstrated that half of patients with AERD were grouped in the fourth quartile, which represented the highest uLTE_4 concentrations (Table II). In contrast, almost half of patients with ATA were located in the first quartile, which represented the lowest uLTE_4 concentrations. Taking the lowest interval as a reference category, any rise in uLTE_4 concentrations significantly increased the odds of AERD diagnosis versus ATA diagnosis. Therefore, uLTE_4 concentrations of 342.0-780.0 pg/mg creatinine increased the odds by 80%, whereas uLTE_4 concentrations >780.0 pg/mg creatinine increased the likelihood of AERD diagnosis 5-fold.

The uLTE_4 concentration, which best discriminated patients with AERD from patients with ATA, was calculated using the ROC curve at the threshold of 800.0 pg/mg creatinine (Figure 2). The AUC was 0.7; 95% confidence interval (CI): 0.66-0.74. The sensitivity and specificity of the test were 49% and 81%, respectively. Its PPV and NPV were 72% and 61%, respectively. After taking into account the prevalence of AERD in the population of asthmatics (7%),¹⁹ the PPV and NPV were 16% and 96%, respectively. The threshold uLTE_4 value proved to be very close to the value, which designated the highest quartile of uLTE_4 concentrations in HC (780.0 pg/mg creatinine). Therefore, in further analysis, the uLTE_4 concentration at the level of 800.0 pg/mg creatinine was used as the cutoff value above which the likelihood of AERD diagnosis was evidently increased.

The impact of the urinary LTE_4 concentration on AERD diagnosis as an added variable to clinical parameters

Using logistic regression procedures, clinical parameters were analyzed to assess their association with AERD diagnosis. Univariate analysis showed that the outcome variable was related to 15 variables (Table III).

Multiple logistic regression analysis (backward elimination) identified the presence of nasal polyps, upper airway symptoms, treatment with nasal corticosteroids, number of asthma exacerbations requiring medical intervention, $\text{FEV}_1\%$ predicted, and age of asthma onset as independent predictors of AERD diagnosis (Table IV). None of the other analyzed variables were statistically important in this model (model A). uLTE_4 concentration was not taken into consideration in this step.

To estimate an independent impact of increased uLTE_4 concentrations on AERD diagnosis, this variable was added to model A, and model B was created (Table IV). In model B, uLTE_4 concentrations ≥ 800.0 pg/mg creatinine significantly and independently increased the likelihood of AERD diagnosis after consideration of the remaining parameters. However, inclusion of this variable into the model somewhat decreased the odds ratio for upper airway symptoms and nasal polyps, whereas asthma exacerbations lost their statistical significance.

To determine whether the increased uLTE_4 concentration enhanced the prediction of AERD diagnosis beyond the level predicted by the clinical parameters alone, scores for models A and B were calculated based on logistic regression coefficients and compared by ROC analysis. Model B had a slightly better discriminative accuracy (AUC 0.867; 95% CI 0.831-0.897) than

TABLE I. Clinical characteristics of the studied groups

Variable	AERD (n = 247)	ATA (n = 239)	HC (n = 95)	P value
Female, n (%)	169 (68.4)	167 (69.9)	53 (56.3)	.039
Age (y), mean (SD)	49.3 (12.9)	49.3 (14.8)	45.9 (13.4)	.058
Age at onset of asthma (y), mean (SD)	30.8 (11.9)	33.8 (17.4)	NA	.03
Asthma duration (y), mean (SD)	18.8 (11.8)	15.3 (13.8)	NA	<.001
BMI (kg/m ²), mean (SD)	26.8 (5.0)	26.9 (5.1)	NM	.86
Asthma exacerbations requiring medical intervention* (number/year), median (IQR)	0.26 (0.57)	0.04 (0.29)	NA	<.001
Hospitalizations in ICU due to asthma, whenever, n (%)	52 (21.3)	19 (8.0)	NA	<.001
Treatment with oral CS, whenever, n (%)	65 (26.3)	39 (16.3)	NA	.017
Upper airway symptoms, whenever, n (%)	233 (95.5)	108 (49.3)	NA	<.001
Upper airway symptoms duration (y), mean (SD)	16.7 (12.6)	7.0 (11.4)	NA	<.001
Nasal polyps, whenever, n (%)	191 (78.6)	46 (20.9)	NA	<.001
Polypectomies, whenever, n (%)	165 (67.6)	44 (19.3)	NA	<.001
Treatment with nasal CS, whenever, n (%)	212 (86.9)	84 (38.5)	NA	<.001
FEV ₁ % predicted, mean (SD)	80.0 (19.9)	87.0 (19.8)	NM	<.001
FEV ₁ % FVC, mean (SD)	69.0 (11.0)	74.9 (10.6)	NM	<.001
FEV ₁ % FVC after bronchodilator, mean (SD)	68.1 (10.9)	71.2 (10.4)	NM	<.001
Positive skin prick test responses, n (%)	152 (62.6)	146 (67.3)	0 (0.0)	.289†
Total IgE concentration (IU/mL), median (IQR)	88.0 (158.2)	84.6 (175.3)	25.9 (52.7)	<.001‡
Blood eosinophil count (cells/mL), median (IQR)	294 (440)	207 (345)	131.0 (141)	<.001§

AERD, Aspirin-exacerbated respiratory disease; ATA, aspirin-tolerant asthma; BMI, body mass index; CS, corticosteroids; FEV₁, forced expiratory volume in the 1 second; FVC, forced vital capacity; HC, healthy control; ICU, intensive care unit; IQR, interquartile range; NA, not applicable; NM, not measured.

*Adjusted to asthma duration.

†AERD vs ATA.

‡Kruskal-Wallis test, statistical difference between AERD vs HC ($P < .001$), ATA vs HC ($P < .001$).

§Kruskal-Wallis test, statistical difference between AERD vs HC ($P < .001$), ATA vs HC ($P = .02$), AERD vs ATA ($P = .002$).

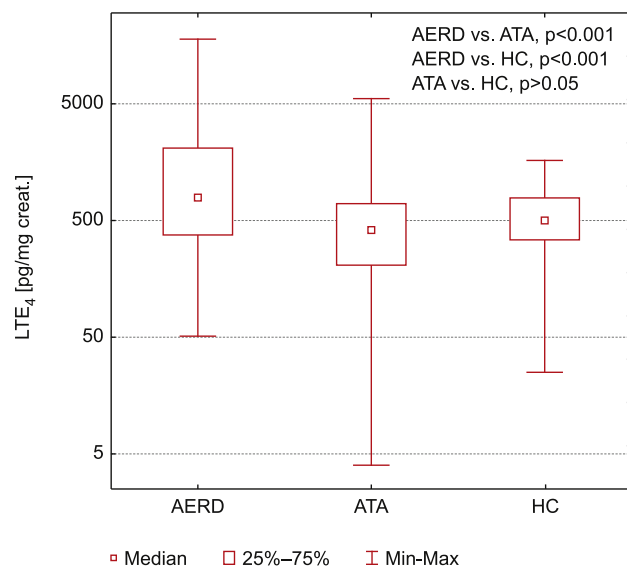


FIGURE 1. Urinary LTE₄ concentrations in patients with AERD, ATA, and HC. LTE₄ concentration expressed by using the log scale. AERD, Aspirin-exacerbated respiratory disease; ATA, aspirin-tolerant asthma; HC, healthy control; LTE₄, leukotriene E₄.

model A (AUC 0.853 95% CI 0.816-0.885) (diff. = 0.014, $P = .036$) (Figure 3).

Additional analysis of differences between the scores by asthma groups revealed the minor shift to the right in the median difference in patients with AERD. In patients with ATA, the

median score of model A (without uLTE₄ concentration) was slightly higher compared with model B (Table V).

DISCUSSION

The results of our study demonstrated that the diagnostic value of LTE₄ measurement in morning urine spot collection using the ELISA method was too weak to be a single parameter capacitating AERD diagnosis in patients with asthma. Clinical parameters such as nasal polyps, upper airway symptoms, nasal corticosteroid treatment, asthma exacerbations, FEV₁% predicted, and age of asthma onset proved to be independent predictors of AERD diagnosis. The addition of increased uLTE₄ concentration to the set of clinical parameters only slightly, though significantly, enhanced the prediction of AERD diagnosis beyond discrimination based on clinical parameters.

Consistent with previous studies, we found that uLTE₄ concentrations were significantly higher in patients with AERD than in patients with ATA and HC.⁷⁻¹³ Although we included a larger number of subjects than previous studies, the considerable overlap between the groups still existed, making it impossible to use uLTE₄ measurement as a diagnostic tool in AERD. However, any rise in the uLTE₄ concentration increased the chance of AERD diagnosis versus ATA diagnosis. Elevated uLTE₄ concentration was an independent predictor, which increased almost 2 times the odds of such diagnosis after adjusting for a set of clinical parameters. In our study, spot uLTE₄ concentration equaling 800.0 pg/mg creatinine measured by ELISA best discriminated patients with AERD from patients with ATA. Despite the observation that the diagnostic accuracy of the test was relatively good (AUC = 0.7), its sensitivity was not satisfactory. A recent meta-analysis, in which the cutoff uLTE₄

TABLE II. Prevalence of AERD and ATA diagnosis by urinary LTE₄ distribution in HC subjects

Asthma group	Urinary LTE ₄ concentrations based on control subjects' distribution		
	Q1 ≤341.0 pg/mg creat.	Q2-Q3 342.0-780.0 pg/mg creat.	Q4 >780.0 pg/mg creat.
AERD, n (%)	52 (21.0)	71 (28.7)	124 (50.2)
ATA, n (%)	108 (45.2)	82 (34.3)	49 (20.5)
OR* (95% CI)	1.0	1.8 (1.14-2.85)	5.3 (3.3-8.4)

AERD, Aspirin-exacerbated respiratory disease; ATA, aspirin-tolerant asthma; CI, confidence interval; HC, healthy control; LTE₄, leukotriene E₄; OR, odds ratio.

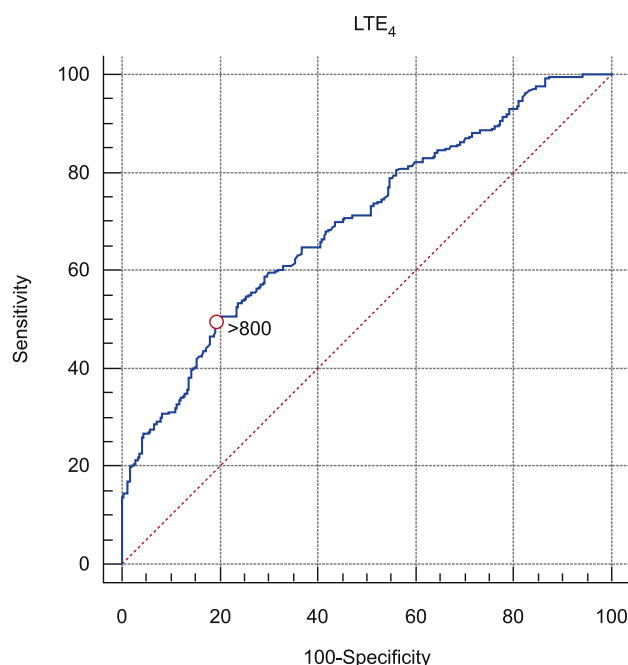
*Odds ratios of AERD vs ATA diagnosis.

concentration measured by the same methodology was 510 pg/mg creatinine, demonstrated similar diagnostic testing accuracy with much better sensitivity.²⁰ The difference as compared with our study could result from different inclusion criteria of other studies.

By applying the same laboratory method, Celejewska-Wójcik et al.²¹ suggested a slightly higher discriminative threshold value of uLTE₄ concentration; however, the test proved to have better diagnostic accuracy and better sensitivity than in our study. Such discrepancies may arise from differences in size of studied populations and their clinical characteristics. All the patients who participated in the study by Celejewska-Wójcik et al.²¹ had chronic rhinosinusitis requiring sinus surgery. However, only half of the ATA group had asthma, whereas all AERD ones were asthmatics. In our study, all patients had asthma presenting a broad profile of both asthma phenotypes. They differed in terms of asthma severity, exacerbations, bronchial obstruction, upper airways involvement, and blood eosinophilia, which was consistent with the results of large population-based studies and meta-analysis.^{2,22-24} Otherwise, the AERD group itself is not homogeneous in respect of clinical presentation and uLTE₄ concentrations.²⁵

Increased uLTE₄ excretion was termed by Higashi et al.¹¹ as hyperleukotrienuria and the cutoff uLTE₄ concentration was suggested as the value exceeding the third standard deviation above the mean concentration of HC subjects. Because a different laboratory method was used, a comparison with our study was difficult. Based on their results, overproduction of CysLTs was not strictly associated with aspirin hypersensitivity itself, but rather with the presence of chronic rhinosinusitis with nasal polyps. Thus, the comparison of the AERD and ATA groups in which upper airways are involved may result in a bias of the test performance.

To eliminate the potential variability in uLTE₄ production, Divekar et al.²⁶ evaluated 24-hour uLTE₄ excretion estimated by liquid chromatography-mass spectrometry (LC-MS). The cutoff uLTE₄ values that discriminated subjects with a history of aspirin sensitivity from tolerant ones and challenge-confirmed aspirin sensitive subjects versus those not confirmed had considerably better sensitivity and specificity than in our study. Despite limitations stipulated by the authors of this study, one may conclude that 24-hour uLTE₄ measurement using LC-MS is the most sensitive laboratory method in diagnosing aspirin hypersensitivity in asthma patients. Indeed, our previous study comparing spot uLTE₄ concentrations in HCs by LC-MS and ELISA methods

**FIGURE 2.** ROC curve for urinary LTE₄ concentrations discriminating patients with AERD from patients with ATA. The discriminative value of LTE₄ equaled 800.0 pg/mg creatinine, AUC = 0.7; 95% CI: 0.66-0.74, sensitivity = 49%, specificity = 81%, PPV = 72%, NPV = 61%. AERD, Aspirin-exacerbated respiratory disease; ATA, aspirin-tolerant asthma; AUC, area under the curve; LTE₄, leukotriene E₄; NPV, negative predictive value; PPV, positive predictive value; ROC, receiver operating characteristic.

demonstrated nearly 10-fold overestimation of the lowest uLTE₄ concentrations by the latter method.²⁷ This could result from interferences in crude urine samples with the immunoassay. However, in clinical practice, morning spot urine collection is more convenient than 24-hour collection. Moreover, a readily available and inexpensive ELISA seems satisfactory enough for ascertainment of uLTE₄, particularly at higher concentrations.²⁷

Because the measurement of a spot uLTE₄ concentration did not offer satisfactory diagnostic value as a single parameter predicting AERD diagnosis in patients with asthma, we evaluated the utility of clinical parameters in this respect. Among variables associated with AERD diagnosis, those related to upper airway involvement, such as upper airway symptoms, nasal polyps, and nasal corticosteroid treatment, were the most effective and independent predictors of AERD. In large cohorts of patients with AERD, the prevalence of chronic sinusitis ranged from 77.9% to 82.4%, whereas the presence of nasal polyps varied from 60.0% to 99.0%, depending on the diagnostic methods used.^{1,2,22,24,28} The recent meta-analysis estimated the prevalence of AERD among patients with chronic rhinosinusitis and nasal polyps to be 8.7% and 9.7%, respectively.¹⁹ In our study, upper airway symptoms, which were surrogates for chronic rhinosinusitis, and the associated nasal corticosteroid treatment were declared by 95.5% and 86.9% of patients with AERD, respectively, and they were approximately 2-fold more frequent than in the ATA group. The presence of upper airway symptoms increased the odds of AERD diagnosis 4.1 times. Nasal polyps were 4-fold

TABLE III. Odds of AERD diagnosis with respect to potential predictive factors estimated by univariate regression analysis

Variable	OR	95% CI	P value
Age at onset of asthma (y), mean (SD)	0.986	0.974-0.999	.029
Asthma duration (y), mean (SD)	1.02	1.01-1.04	.005
BMI (kg/m ²)	1.00	0.99-1.01	.84
Asthma exacerbations requiring medical intervention* (number/year), median (IQR)	1.33	1.08-1.63	.007
Hospitalizations in ICU due to asthma exacerbations, whenever (Yes/No)	2.92	1.67-5.13	<.001
Treatment with oral CS, whenever (Yes/No)	1.71	1.09-2.68	.018
Upper airway symptoms, whenever (Yes/No)	18.14	9.13-36.04	<.001
Upper airway symptoms duration (y), mean (SD)	1.07	1.05-1.09	<.001
Nasal polyps, whenever (Yes/No)	13.89	8.88-21.75	<.001
Polypectomies (Yes/No)	8.73	5.71-13.37	<.001
Treatment with nasal CS, whenever (Yes/No)	10.57	6.66-16.78	<.001
FEV ₁ % predicted (%)	0.98	0.97-0.99	<.001
FEV ₁ % FVC, mean (SD)	0.95	0.93-0.97	<.001
FEV ₁ % FVC after bronchodilator <70% (Yes/No)	1.95	1.28-2.95	.002
Positive skin prick test responses (Yes/No)	0.93	0.64-1.34	.685
Total IgE concentration (log ₂) (IU/mL)	1.001	0.90-1.11	.979
Blood eosinophil count >400 cells/mL (Yes/No)	1.89	1.29-2.78	.001
Urinary LTE ₄ concentration (pg/mg creat.) ≥800 pg/mg creat. (Yes/No)	4.05	2.70-6.08	<.001

AERD, Aspirin-exacerbated respiratory disease; BMI, body mass index; CI, confidence interval; CS, corticosteroids; FEV₁, forced expiratory volume in the 1 second; FVC, forced vital capacity; ICU, intensive care unit; IQR, interquartile range; LTE₄, leukotriene E₄; OR, odds ratio.

*Adjusted to asthma duration.

TABLE IV. Odds of AERD diagnosis with respect to potential predictive factors estimated by multivariate regression analysis

Variable	Model A			Model B		
	OR	95% CI	P value	OR	95% CI	P value
Age at onset of asthma (y)	0.98	0.96-0.995	.013	0.98	0.96-0.996	.014
Asthma exacerbations requiring medical intervention* (number/year)	1.2	1.01-1.41	.04	1.18	0.99-1.40	.065
Upper airway symptoms, whenever (Yes/No)	4.1	1.58-10.6	.004	3.77	1.44-9.84	.007
Nasal polyps, whenever (Yes/No)	6.0	3.5-10.3	<.001	5.23	3.02-9.08	<.001
Treatment with nasal CS, whenever (Yes/No)	2.18	1.08-4.4	.03	2.31	1.14-4.67	.02
FEV ₁ % predicted (%)	0.98	0.97-0.995	.007	0.98	0.97-0.995	.007
Urinary LTE ₄ concentration ≥800.0 pg/mg creat. (Yes/No)				1.84	1.08-3.14	.02

AERD, Aspirin-exacerbated respiratory disease; CI, confidence interval; CS, corticosteroids; FEV₁, forced expiratory volume in the 1 second; LTE₄, leukotriene E₄; OR, odds ratio.

*Adjusted to asthma duration.

more frequent in the AERD group, and they were diagnosed in 78.6% of this population. Their presence was the most predictive, independent clinical parameter, which increased the odds of AERD diagnosis 6 times. In the Korean study, nasal polyposis and chronic rhinosinusitis were the 2 of 4 clinical parameters showing a significant, independent association with AERD, and they increased the odds of the diagnosis 2.4 and 4.28 times, respectively.²⁹ Chronic rhinosinusitis and nasal polyps were independent predictors for AERD in patients with asthma in Turkey.³⁰ Nasal polyps were also independently associated with AERD diagnosis in an Australian population of asthmatics.³¹ Such consistent results originating from different populations provide convincing proof that upper airway involvement and particularly nasal polyposis constitute strong predictive factors for AERD diagnosis.

Asthma exacerbations requiring medical intervention were another independent predictor, which increased AERD diagnosis by 20% in our study. Their occurrence was over 6 times more frequent in patients with AERD than in patients with ATA.

Higher frequency of asthma-related health care use by patients with AERD than by patients with ATA was also found in large cohort studies carried out in different populations of asthmatics.^{2,23,31,32} Asthma attacks were predictive of AERD diagnosis in Australian patients with asthma.³¹ This confirms that AERD comprises high-risk asthmatics, which require more intensive care and follow-up. Therefore, it is recommended for these patients to remain under the care of experienced health care providers.³³

The Epidemiology and Natural History of Asthma: Outcomes and Treatment Regimens (TENOR) study revealed that patients with AERD can be distinguished from aspirin tolerant ones by more severe bronchial obstruction,² although this was not confirmed by other population-based studies.^{23,32} In our study, patients with AERD had significantly lower FEV₁% predicted and FEV₁/FVC ratio than patients with ATA, and lower FEV₁ was the next independent predictor for AERD diagnosis. In addition, the FEV₁/FVC ratio under 0.7 persisted after bronchodilator administration and was still lower in the AERD

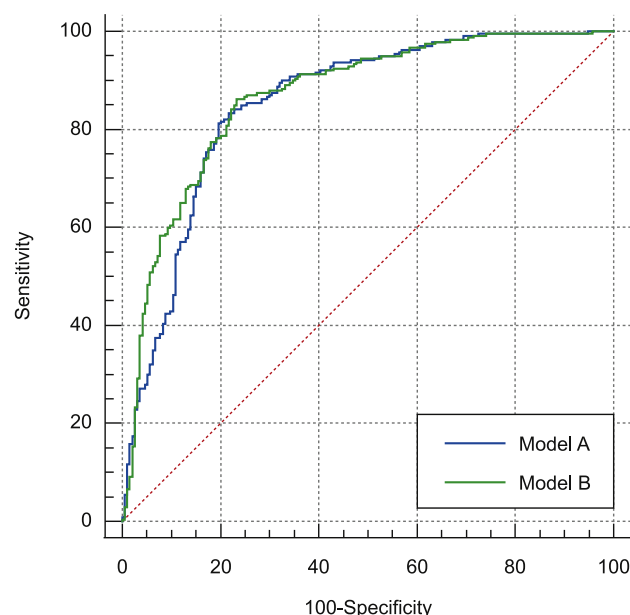


FIGURE 3. Receiving operating characteristic curve of scores A and B. AUC for model A = 0.853, 95% CI 0.816-0.885; AUC for model B = 0.867, 95% CI 0.831-0.897, difference = 0.014, $P = .036$. AUC, Area under the curve; CI, confidence interval.

TABLE V. Comparison of scores of models A and B for patients with AERD and ATA

	Score A		Score B		<i>P</i> value*
	Median	Q1-Q3	Median	Q1-Q3	
AERD	64.0	58.9-68.5	65.2	57.6-69.5	<.001
ATA	39.2	24.4-50.4	39.0	23.7-50.2	.002

AERD, Aspirin-exacerbated respiratory disease; ATA, aspirin-tolerant asthma.

*Wilcoxon signed rank sum test.

group, which might suggest more advanced remodeling of the bronchi in these patients. On the basis of pulmonary function test results, the TENOR study investigators hypothesized that AERD was associated with aggressive remodeling of the airways.² However, when high-resolution computed tomography was applied, no significant difference was found between patients with AERD and patients with ATA.³⁴

Age at onset of asthma was another independent predictor negatively associated with AERD diagnosis in our study. It is widely accepted that asthma in patients with AERD usually begins in the third or fourth decade of life.¹ The mean age of asthma onset in patients with AERD participating in our study was lower than that in the ATA group corroborating with the results obtained in other populations of asthmatics.^{29,30} These data suggest that the later the age of asthma onset, the less chance of AERD diagnosis. This is also in agreement with the results of a study by Dursun et al.,³⁵ who demonstrated that the age of patients under 40 increased the odds of AERD diagnosis.

Our study is the first to evaluate the diagnostic utility of a spot uLTE₄ measurement in combination with clinical parameters to predict AERD diagnosis in patients with asthma. Chang et al.²⁹ evaluated only clinical parameters, of which history of aspirin

hypersensitivity, chronic rhinosinusitis, nasal polyps, and PC₂₀ methacholine proved to be independently associated with AERD. Two of them were also predictive in our study. We did not analyze history of aspirin hypersensitivity and bronchial hyperreactivity because all patients with AERD had established asthma with aspirin hypersensitivity, which was confirmed by positive provocation challenge in the majority of them.

The strength of our study consists in a careful clinical evaluation of asthmatics, the results of which were included in the prediction model. Its limitation can be the fact that 20% of patients with AERD did not have aspirin hypersensitivity confirmed by aspirin challenge and their assignment was based on their medical history. However, their clinical presentation was highly typical for AERD and previous reactions caused by ingestion of aspirin and other NSAIDs were well documented. Removal of these severe asthmatics could introduce additional bias in characterization of the AERD phenotype.

In conclusion, our study revealed that, in patients with asthma, the performance of a set of typical clinical parameters has much better accuracy in the prediction of AERD diagnosis than the measurement of a spot uLTE₄ concentration alone. The addition of the uLTE₄ result ≥ 800.0 pg/mg creatinine measured by ELISA to the model comprising clinical parameters only slightly, though statistically significantly, enhances the accuracy of AERD diagnosis. Assuming the prevalence of AERD in asthma population, uLTE₄ concentration demonstrated a high NPV for AERD diagnosis and could be useful in delaying or cancelling aspirin challenge if clinical status of asthmatic does not allow drug provocation. The application of such a model might prove helpful as a screening procedure in clinical settings not performing aspirin challenges to distinguish patients with asthma who are at a higher risk of AERD diagnosis.

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