

Elevated urinary leukotriene E₄ excretion in asthma: a comparison of HPLC-mass spectrometry and ELISA

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Measurement of urinary leukotriene E₄ (uLTE₄) estimates systemic production of cysteinyl leukotrienes. Increased uLTE₄ is often found in asthma, urticaria and atopic dermatitis

(1–3). It is a hallmark of aspirin-induced asthma (AIA) and aspirin-sensitive urticaria (4, 5). As a result of a low concentration, uLTE₄

has to be measured using enzymatic immunoassay (ELISA) or HPLC-mass spectrometry. Unpurified urine samples (6) have unspecific interaction with ELISA assay, overestimating uLTE₄. Purification of urine, by affinity extraction with monoclonal antibody, solid phase extraction or liquid chromatography, can improve specificity, but uLTE₄ recovery is quite variable and < 70%.

We ascertained uLTE₄ using a method of affinity purification of large (25 ml) volume of urine, added deuterated LTE₄ as internal standard, and subsequently carried out measurements using HPLC-mass spectrometry (HPLC-MS, LCQ Advantage, Thermo Finnigan, San Jose, CA, USA). Alternatively, we performed estimations with ELISA (Cayman Chemical Co., Ann Arbor,

MI, USA) in unpurified urine (dilution 1 : 10 or 1 : 20). Duplicated experimental urine samples to which known concentrations of LTE₄ had been added were tested along with 174 clinical samples by both methods.

In the experimental urine samples, within uLTE₄ range from 0 to 200 pg/ml, uLTE₄ measurement better performed using HPLC-MS method ($r^2 = 0.999$) than using ELISA ($r^2 = 0.857$), Fig. 1. At higher concentrations of uLTE₄ (500, 1000 and 2000 pg/ml) ELISA assay satisfactorily determined the concentration ($r^2 = 0.98$). Morning samples of urine were collected from adult healthy subjects ($n = 50$, 21 males, mean age 31.4 [range 21–60] years) and asthmatic patients ($n = 124$, 46 males, mean age 44.5 [21–66] years). Urinary leukotriene E₄ was recalculated on milligram of creatinine. Spearman rank correlation between the methods was $R = 0.636$. There was a significant difference between healthy males and females. When HPLC-MS method was used, females excreted twice as much as males of uLTE₄ (42.4 [95% confidence interval 31.1–53.8] vs 23.7 [18.5–28.9] pg/mg creatinine; $P < 0.01$). This gender difference was not observed using ELISA, average values of which were overestimated approximately tenfold

(females: 280.3 ± 43 vs males: 392 ± 62 pg/mg creatinine). In the asthmatic subjects, average uLTE₄ was higher than in the healthy subjects (HPLC-MS: 354.0 [210–498], ELISA: 674.9 [527–823] pg/mg creatinine). Forty-one asthmatics (18 males) had AIA, confirmed by the aspirin challenge test. These patients' uLTE₄ was the highest (HPLC-MS: 808.2 [415.5–1201] and ELISA: 1364.3 [1002–1726] pg/mg creatinine). Urinary leukotriene E₄ in clinical samples followed normal distribution after logarithmic transformation. Analysis of log-transformed uLTE₄ for HPLC-MS proved significance of differences between healthy subjects and asthmatics and their subgroups, and between AIA and asthmatics who tolerated aspirin well. However, with ELISA method no difference between healthy subjects and asthmatics, who tolerated aspirin well, was noted. At the threshold of 48.6 mg/mg creatinine (HPLC-MS), 81.9% asthmatic subjects could be distinguished from healthy subjects (80.6% sensitivity, 63.2% specificity). ELISA at threshold of 187 pg/mg creatinine showed worse test performance (62.2% correctly distinguished, 80.2% sensitivity, 21.6% specificity). Interestingly, HPLC-MS identified 82.6% AIA from the asthmatics group (threshold 71 pg/mg creatinine, 91.3%

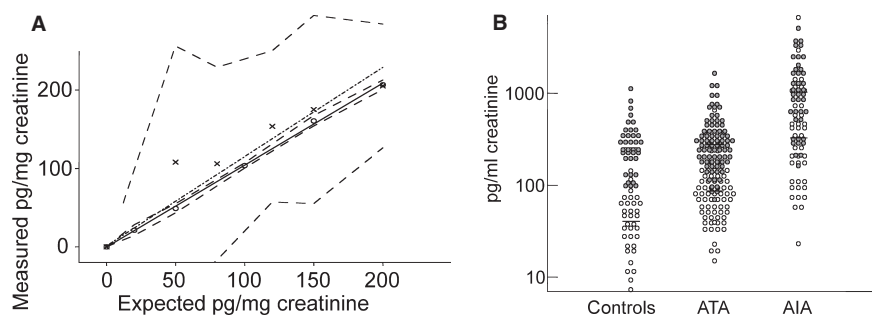


Figure 1. Correlation between measured uLTE₄ and expected values over the range 0 to 200 pg/mg creatinine in duplicated experimental urine samples (A). Circles – HPLC-MS, crosses – ELISA. 95% confidence intervals for linear regressions marked by dashed lines. (B) ELISA (grayed) and HPLC-MS uLTE₄ (open circles) in study subjects by groups. Mean values marked by horizontal bars.

sensitivity, 44.3% specificity), while with ELISA the results were not worse (86.8% identified, threshold 274 pg/mg creatinine, sensitivity 90.2%, specificity 50.6%).

In conclusion, a readily available ELISA measurement of uLTE₄ using unpurified urine samples offers uncompromising prediction of AIA, not different from HPLC-MS. However, to demonstrate elevated uLTE₄ in adult asthmatics or gender differences in cysteinyl leukotrienes systemic production, a more sensitive and specific method of HPLC-MS is required.

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References

1. Rabinovitch N. Urinary leukotriene E₄. *Immunol Allergy Clin North Am* 2007; **27**:651–664.
2. Mastalerz L, Setkowicz M, Sanak M, Szczeklik A. Hypersensitivity to aspirin: common eicosanoid alterations in urticaria and asthma. *J Allergy Clin Immunol* 2004; **113**:771–775.
3. Vachier I, Kumlin M, Dahlen SE, Bousquet J, Godard P, Chanez P. High levels of urinary leukotriene E₄ excretion in steroid treated patients with severe asthma. *Respir Med* 2003; **97**:1225–1229.
4. Nasser S, Lee TH. Leukotrienes in aspirin-sensitive asthma. In: Szczeklik A, Gryglewski RJ, Vane JR. *Eicosanoids, aspirin and asthma*. New York: Marcel Dekker Inc, 1998: 317–336.
5. Gaber F, Daham K, Higashi A, Higashi N, Gulich A, Delin I et al. Increased levels of cysteinyl-leukotrienes in saliva, induced sputum, urine and blood from patients with aspirin-intolerant asthma. *Thorax* 2008; **63**:1076–1082.
6. Kumlin M, Stensvad F, Larsson L, Dahlen B, Dahlen SE. Validation and application of a new simple strategy for measurements of urinary leukotriene E₄ in humans. *Clin Exp Allergy* 1995; **25**:467–479.