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A new, sensitive LC-MS/MS assay for quantification of uric acid in urine

M. van der HAM[1], B.H.C.M.T. PRINSEN[1], I.M.L.W. KEULARTS[2,3], J. BIERAU[3], T.J. de KONING[1] and M.G.M. de SAIN-van der VELDEN[1]

Introduction

Inborn errors of purine metabolism are serious hereditary disorders, which should be suspected in patients who present with neonatal seizures, failure to thrive, recurrent infections, neurological deficit, renal disease, and self-mutilation. Investigations may start with uric acid (UA) determination in urine and plasma. UA, the final product of purine metabolism in humans, may be altered not only in purine inborn error of metabolism, but also in other pathological and clinical conditions and clinical conditions (1). Urinary UA (UUA) is related to plasma UA concentrations which makes UUA a diagnostically important biomarker for screening for inborn errors in purine metabolism (2, 3). The frequently used enzymatic assays (4, 5) for UA measurements are prone to interferences. We therefore developed and validated a LC-MS/MS method for quantification of UUA and compared our method to three other quantitative assays (one HPLC assay and two enzyme-based assays) (2, 4, 5).

Materials and Methods

Urine samples for reference values were obtained from 1032 individuals (age 0 – 72 years), who were examined in our hospital for non IEM-related reasons. Urine was collected without any restriction and stored at -20 °C until analysis. In addition, urine samples of seven patients with different disorders in purine metabolism were investigated: Lesch-Nyhan syndrome (n=4), Xanthine Dehydrogenase (XDH) deficiency (n=2), Purine Nucleotide Phosphorylase (PNP) deficiency (n=1) and a urine sample of a patient with fructose-1,6-biphosphatase deficiency.

UA was analyzed after sonification, dilution and centrifugation of urine samples. 1,3-$^{15}N_2$-UA was used as internal standard. LC-MS/MS was performed in negative elektrospray ionization mode with multiple reaction monitoring of transitions m/z 167.0 → 124.0 (UA) and m/z 169.0 → 125.0 ($^{15}N_2$-UA). Correlation studies for LC-MS/MS, HPLC-PDA (Waters) and two enzymatic assays (Vitros URIC slide and Beckman Coulter Synchron LC method (uricase-peroxide method)) were made. Interference of ascorbic acid on the enzymatic as well as on the LC-MS/MS method was studied.

Department of Metabolic and Endocrine Diseases[1], University Medical Center Utrecht; Department of Clinical Chemistry[2], Meander Medical Center, Amersfoort and Department of Clinical Genetics[3], Academisch Ziekenhuis Maastricht

Results

Limits of detection and limit of quantification of UA with the new LC-MS/MS method were 0.2 and 0.6 µmol/L, respectively. Intra- and inter assay variations of UA were 3.6 % and 7.0 %, respectively. Linearity was tested between 0-830 µmol/L (r=0.9996). Results from the HPLC-PDA assay showed an acceptable correlation (r^2=0.9886) with the LC-MS/MS method. A major systematic difference (Bland-Altman plot) of 20%, however, was observed (figure 1a) probably due to differences in calibration. The correlation between the uricase assay and LC-MS/MS was good up to a concentration of 2000 µM; UUA > 2000 µM gave major discrepancies between the two assays (figure 1b). Analysis of UA-spiked samples (with UA calibrators up to 6500 µmol/L) by LC-MS/MS gave recoveries between 98.3 -104.5%. Urine samples from patients with

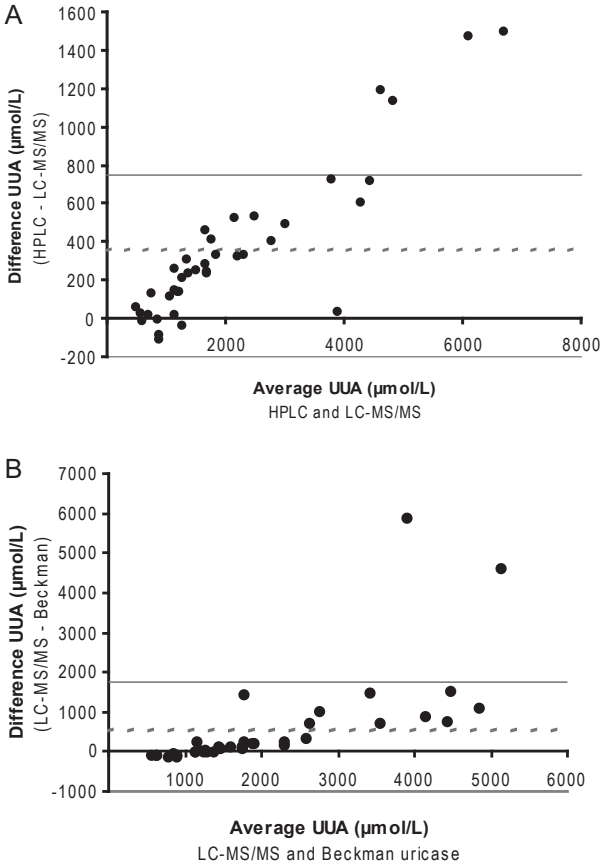

Figure 1. Method comparison by Bland-Altman plots for urinary UA assays**.** (A) Correlation between the LC-MS/MS and HPLC method and (B) between the LC-MS/MS and uricase method (Synchron LX, Beckman Coulter). Thick dashed lines indicate mean Y-values; thin dashed lines indicate 1 SD.

Table 1. Uric acid (UA) excretion in urine samples from patients with inborn errors of metabolism affecting UA metabolism analyzed by LC-MS/MS. XDH: Xanthine Dehydrogenase deficiency; PNP: Purine Nucleotide Phosphorylase deficiency. Information related to age and creatinine excretion is missing for samples 1, 2, 6, and 7 (*). ↑, increased regarding to age-related reference values; ↓, decrease regarding to age-related reference values.

Sample	Disorder	Age	Urinary UA excretion µmol/L	mmol/ mol creatinine)	
1	Lesch-Nyhan syndrome	*	5191	*	↑
2	Lesch-Nyhan syndrome	*	11539	*	↑
3	Lesch-Nyhan syndrome	13 month	7594	3452 (ref. 308-1711)	↑
4	Lesch-Nyhan syndrome	18 months	2611	2611 (ref. 308-1711)	↑
5	XDH deficiency	2.5 year	14	18 (ref. 258-1607)	↓
6	XDH deficiency	*	< 0.6	*	↓
7	PNP deficiency	*	90	*	↓
8	Fructose-1,6- -biphosphatase deficiency	1 day	17784	5558 (ref. 508-2425)	↑

a defect in purine metabolism (HPRT, XDH and PNP) and a patient with fructose-1,6-biphosphatase deficiency were analyzed with the new LC-MS/MS assay and results were compared with age-related reference values (table 1). Ascorbic acid concentrations > 60 nmol/l were shown to interfere in the uricase assay (>5% decrease in UA concentration) but not in the LC-MS/MS assay.

Conclusions

A LC-MS/MS method has been developed for routine determination of urinary UA. Analysis of UA-spiked samples and UA calibrators show that the LC-MS/MS method is very accurate. The enzymatic assay shows major discrepancies with the LC-MS/MS method at higher UA concentrations, and is therefore not the assay of choice for diagnostic purposes related to diagnosis of inborn errors of purine metabolism.

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Cardiac biomarkers in dialysis patients: variations during a six month follow up

L. JACOBS[1], J. van de KERKHOF[3], A. MINGELS[1], V. KLEIJNEN[1], F. van der SANDE[2], W. WODZIG[1], J. KOOMAN[2] and M. van DIEIJEN-VISSER[1]

Introduction

Cardiovascular complications have a high prevalence in patients suffering from end-stage renal disease (ESRD). The main cause of death in these patients is accounted for by cardiovascular events and over 55% of dialysis patients suffer from congestive heart-failure (1). Considering the high incidence of cardiovascular disease, there is a need for accurate and sensitive biomarkers for both diagnosis and risk stratification in these patients. Over the years cardiac troponin (cTn) has emerged as a potential diagnostic and prognostic cardiac biomarker (2). The levels of cTn might, however, be influenced by a decrease in renal function, which can impair its prognostic and diagnostic functionality (3). For example, the presence of elevated

Department of Clinical Chemistry[1], Department of Internal Medicine, Division of Nephrology[2], University Hospital Maastricht and Department of Internal Medicine, Division of Nephrology[3], Bernhoven Hospital Veghel