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.

Acknowledgement

The authors would like to thank dr. Nanda Verhoeven, dr. Bert Dorland and prof. dr. Ruud Berger for critically reading the manuscript.

References

1. Simoni RE, Gomes LN, Scalco FB, Oliveira CP, Aquino Neto FR, Oliveira ML de. Uric acid changes in urine and plasma: an effective tool in screening for purine inborn errors of metabolism and other pathological conditions. J Inherit Metab Dis 2007; 30: 295-309.
2. Duran M, Dorland L, Meuleman EE, Allers P, Berger R. Inherited defects of purine and pyridimide metabolism: laboratory methods for diagnosis. J Inher Metab Dis 1997; 20: 227-236.
3. Terkeltaub R, Bushinski DA, Becker MM. Recent developments in our understanding of the renal basis of hyperuricemia and the development of novel anti hyperuricemic therapeutics. Arthritis Res Ther 2006; 8 (Suppl 1): S4.
4. Fossati P, Prencipe L, Bert G. Use of 3,5-dichloro-2-hydroxybenzenesulfonic acid/4-aminophenazone chromogenic system in direct enzymatic assay of uric acid in serum and urine. Clin Chem 1980; 26: 227-231.
5. Gochman N, Schmitz JM**.** Automated determination of uric acid, with use of a uricase-peroxidase system. Clin Chem 1971; 17: 1154-1159.

Ned Tijdschr Klin Chem Labgeneesk 2008; 33: 176-178

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

levels of cTn in ESRD patients, in the absence of apparent cardiac damage, frustrates the diagnosis of an acute myocardial infarction (AMI). Recent NACB guidelines have addressed this issue and suggest that for patients with chronically elevated levels of cTn changes in cTn (>20%) 6-9 hours after the onset of clinical symptoms are indicative of an AMI (4). However, they do not address changes in cTn levels in the absence of clinical symptoms, although ESRD studies using serial measurements have shown that cTn concentrations vary significantly over longer periods of time (5, 6). In our study we investigated the intra-individual variation in the cTn serum concentrations of 44 ESRD patients during a 6-month period. In addition, we assessed the performance of two recently developed high-sensitive cTn assays, the pre-commercial high-sensitive cTnT assay (hs-cTnT) (Roche Diagnostics, Mannheim, Germany) and the Architect i2000SR TnI assay (cTnI) (Abbott Diagnostics, Wiesbaden, Germany) in detecting cTn elevations and variations in ESRD patients. A current cTnT assay was included for comparison.

Methods

The patient population consisted of a cohort of 44 chronic hemodialysis patients from the Department of Internal Medicine at the University Hospital Maastricht. The study protocol was approved by the medical ethical review committee of the University Hospital Maastricht (Medical Ethical Committee azM/UM). Blood samples were collected pre-dialysis at the start of the study and subsequently every two months for a period of 6 months. Additional samples were collected when patients were admitted to the hospital. Collected serum samples were stored at -80 °C until analysis.

CTnI was measured on the Architect i2000SR (Abbott Diagnostics, Wiesbaden, Germany) with a lower limit of detection (LOD) of 0.009 µg/L and a 10% coefficient of variation (CV) of 0.032 µg/L. CTnT was measured on the Elecsys 2010 (Roche diagnostics, Mannheim, Germany) using the pre-commercial high-sensitive cTnT (hs-cTnT) assay with a LOD of 0.000 µg/L and the 10% CV level at 0.009 µg/L. CTnT was additionally measured on the Elecsys 2010 using the 4th generation cTnT immunoassay with the LOD at 0.01 µg/L and the 10% CV at 0.03 µg/L (according to the package insert). Reference decision limits (i.e. 99th percentile) were determined in a reference population of 501 apparently healthy persons who participated in a health check program at the University Hospital Maastricht, the Netherlands.

Data analyses were performed using Statistical Package for Social Sciences (SPSS), version 15.0 for Windows (SPSS Inc., Chicago, IL, USA). Reference limits were calculated non-parametrically. Continuous variables are described as median and interquartile range (IQR) or as average and standard deviation (SD). Categorical variables are described as absolute numbers and as percentages.

Results

The ESRD patient population consisted of 30 male and 14 female patients with an average (+/- SD) age of 66 (+/- 10.50) years. Patients have been on dialysis for 30 (+/- 24) months. Within our population 64% of the patients suffered from hypertension, 34% had diabetes and 32% had a history of ischemic heart disease. At baseline, median cTn concentrations (IQR), as detected by the cTnT, hs-cTnT and cTnI assays were 0.03 µg/L (<0.01-0.05), 0.056 µg/L (0.036-0.090) and 0.017 µg/L (0.005- 0.035). Table 1 shows the number of patients exceeding some commonly used analytical cut-off concentrations for each of the cTn assays under investigation.

The biological variability in cardiac biomarker concentrations was measured during a 6-month period. Figure 1 visualizes the differences between the minimum and maximum cTn concentrations measured during the 6-month period for each of the patients. Median (IQR) within-patient variations in cTn concentrations during the study period were 0.038 µg/L (0.019-0.074) for the cTnT assay, 0.021 µg/L (0.011-0.048) for the hs-cTnT assay and 0.018 µg/L (0.007-0.026) for the cTnI assay. During the follow-up an additional number of patients reached cTn levels above the different cut-off levels, as can be seen in table 1.

Table 1. Number of patients having above cut-off cardiac troponin concentrations at baseline and at least once during the six-month follow-up period

Cardiac Troponin assay		LOD	10% CV	99th percentile	AMI cut-off*
Elecsys 2010 troponin T STAT	Baseline	31 (70.5%)	21 (47.7%)	31(70.5%)	21 (47.7%)
(cTnT)	Follow-up	43 (98%)	37 (84%)	43 (98%)	37 (84%)
Cut-off concentration in µg/L		< 0.01	0.03	< 0.01	0.03
Elecsys 2010 High sensitive cTnT	Baseline	44 (100%)	44 (100%)	42 (95%)	42 (95%)
(hs-cTnT)	Follow-up	44 (100%)	44 (100%)	44 (100%)	44 (100%)
Cut-off concentration in µg/L		0.000	0.009	0.016	0.016
Abbot Architect i2000SR cTnI	Baseline	28 (63.6%)	12 (27.3%)	23(52.3%)	12 (27.3%)
(cTnI)	Follow-up	34 (77%)	18 (41%)	32 (73%)	18 (41%)
Cut-off concentration in µg/L		< 0.009	0.032	0.013	0.032

* According to recent NACB guidelines the 99th percentile is the recommended cut-off level in AMI. However, the assay imprecision (%CV) should be ≤ 10% at the 99th percentile (7).

Conclusion

Our results show, for the first time, that virtually all ESRD patients have increased levels of cTn at least once during a 6-month follow up. At baseline 47% of patients had an elevated level of cTnT according to the 4th generation cTnT assay, which is in line with the numbers found in other studies (8). Using the pre-commercial highly sensitive cTnT assay, however, we found that virtually all dialysis patients (95%) had increased levels of cTnT. Serially measuring cTn concentrations during a 6-month follow-up resulted in an additional number of patients showing cTn concentrations above the pre-defined reference values. During follow-up 98%, 100% and 73% of the patients showed cTn levels above the 99th percentile according to the cTnT, hs-cTnT and cTnI assays, respectively.

These findings, although limited by the relatively small sample size, suggest that myocardial release of cTn occurs more often than can be extrapolated from a single (baseline) measurement. Considering the diagnostic and prognostic value of cTn, a significant rise in its concentration should alarm physicians. Increases in cTn levels could represent additional myocardial damage. Tracking longitudinal changes in cTn concentrations can aid in identifying patients with underlying pathologies responsible for the increases in cTn and might be used to prevent further increases.

Additional risk stratification could be provided by the use of highly sensitive cTn assays. Assays with sufficient sensitivity to accurately determine the reference values in a healthy population will improve the detection of 'abnormal' cTn levels in ESRD and other patient populations. More sensitive assays will also allow detection of temporal changes in cTn concentrations which could not be detected to date. In the setting of heart failure, the measurement of previously undetectable levels of cTnT were shown to have important prognostic value (9). Further research is needed to show if such a prognostic value exists in the setting of ESRD and how patients can further benefit from serial measurements using highly sensitive cTn assays.

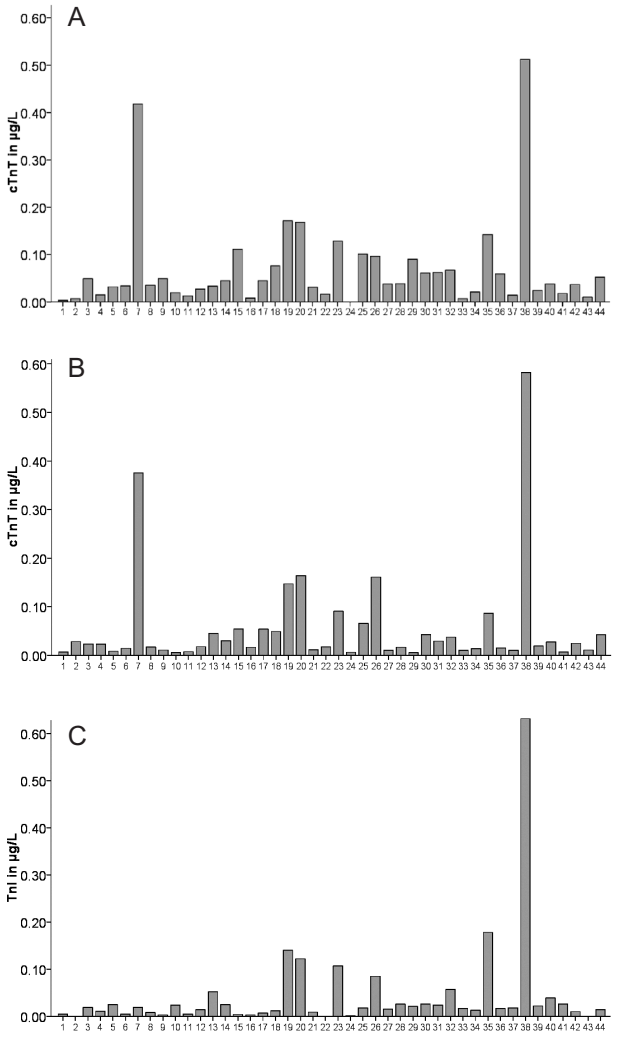

Figure 1. Variation in cTn levels: each line represents the differences between the minimum and maximum cTn concentrations measured during the 6-month period for each of the patients. (A) cTnT-assay (B) hs-c TnT-assay, (C) cTnI-assay.

References

1. U.S. Renal Data System, USRDS 2007 Annual Data Report: Atlas of Chronic Kidney Disease and End-Stage Renal Disease in the United States, National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD, 2007: 192-208.
2. Sommerer C, Giannitsis E, Schwenger V, Zeier M. Cardiac biomarkers in haemodialysis patients: the prognostic value of amino-terminal pro-B-type Natriuretic Peptide and cardiac troponin T. Nephron Clin Pract 2007; 107: c77-c81.
3. Freda BJ, Tang WH, Van Lente F, Peacock WF, Francis GS. Cardiac troponins in renal insufficiency. Review and clinical implications. J Am Coll Cardiol 2002; 40: 2065-2071.
4. Wu AH, Jaffe AS, Apple FS, Jesse RL, Francis GL, Morrow DA, et al. National Academy of Clinical Biochemistry Laboratory Medicine Practice Guidelines: Use of cardiac troponin and B-type Natriuretic Peptide or N-terminal proB-Type Natriuretic Peptide for etiologies other than acute coronary syndromes and heart failure. Clin Chem 2007; 53: 2086-2096.
5. Roberts MA, Fernando D, Macmillan N, Proimos G, Bach LA, Power DA, et al. Single and serial measurements of cardiac troponin I in asymptomatic patients on chronic hemodialysis. Clin Nephrol 2004; 61: 40-46.
6. Miller WL, Hartman KA, Burritt MF, Grill DE, Rodeheffer RJ, Burnett JC, Jr., et al. Serial biomarker measurements in ambulatory patients with chronic heart failure: the importance of change over time. Circulation 2007; 116: 249-257.
7. Apple FS, Jesse RL, Newby LK, Wu AH, Christenson RH, Cannon CP, et al. National Academy of Clinical Biochemistry and IFCC Committee for Standardization of Markers of Cardiac Damage Laboratory Medicine Practice Guidelines: analytical issues for biochemical markers of acute coronary syndromes. Clin Chem 2007; 53: 547-551.
8. Kanderian AS, Francis GS. Cardiac troponins and chronic kidney disease. Kidney Int 2006; 69: 1112-1114.
9. Latini R, Masson S, Anand IS, Missov E, Carlson M, Vago T, et al. Prognostic value of very low plasma concentrations of troponin T in patients with stable chronic heart failure. Circulation 2007; 116: 1242-1249.