Clinical Biochemistry 46 (2013) 1128–1130

Contents lists available at SciVerse ScienceDirect

Clinical Biochemistry

journal homepage: www.elsevier.com/locate/clinbiochem

Short Communication

Measurement of cystatin C in human urine by particle-enhanced turbidimetric immunoassay on an automated biochemistry analyzer

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ARTICLE INFO

Article history:
Received 11 February 2013
Received in revised form 14 April 2013
Accepted 29 May 2013
Available online 12 June 2013

Keywords:
Urinary cystatin C
Cystatin C
Acute kidney injury
Tubular damage
Particle-enhanced turbidimetric immunoassay

ABSTRACT

Background: Cystatin C (CysC), is produced by all the nucleated cells of the human body, is freely filtered by the kidney glomerulus and reabsorbed by the tubules. It is widely accepted that no tubular secretion of CysC occurs. Raised urinary levels are believed to indicate tubular damage.

Methods: We report here the validation of a quantitative assay to measure urinary cystatin C (uCysC) using a commercial CysC kit based on a latex particle-enhanced turbidimetric immunoassay (PETIA), on an automated biochemistry analyzer. The clinical relevance of this assay was tested on several kidney disease patients and a reference range was determined using healthy controls.

Results: The assay is precise (total CV < 4%), and sensitive (limit of quantification = 0.06 mg/dL, and limit of detection = 0.02 mg/L). Calibration is stable for at least 30 days. The assay showed very good linearity over the studied interval (0.02 to 2.25 mg/L). Recovery ranged from 101.62 to 106.49%. The analyte is stable, at 4 °C for at least 2 days, and at 20 °C for 48 h. The upper reference value was 0.12 mg/L Median uCysC concentration in 30 acute kidney injury patients (1.47 mg/L, interquartile range = 0.27–3.87 mg/L) and was significantly higher than that in 25 patients with normal kidney function (0.05, 0.03–0.12; $p < 0.0001$), 30 patients with chronic kidney disease (0.13, 0.05–0.77; $p < 0.0001$) and 15 patients with pre-renal azotemia (0.15, 0.08–0.31; $p < 0.0001$).

Conclusion: Our data indicate that uCysC can be processed on automated biochemistry analyzers and its measurement could easily be added to a standard panel to screen kidney diseases.

Introduction

CysC, an endogenous non-glycosylated protein with a molecular mass of 13 kDa, has been recently investigated as a potential early and sensitive marker of acute kidney injury (AKI). CysC is produced at a constant rate by all nucleated cells and secreted extracellularly shortly after synthesis therefore it is present in all body fluids and tissues [1]. After its secretion, CysC is freely and totally filtered through the glomerulus and reabsorbed by proximal tubular cells where it is completely catabolized and degraded in healthy individuals, with the remaining eliminated with the urine. No tubular secretion of CysC exists; therefore its urinary level is very low in healthy individuals. In cases of tubular injury the urinary levels are expected to rise but the plasmatic level increases only with a decrease in glomerular filtration [2]. uCysC has recently been proposed as a biomarker for AKI and acute tubular necrosis [3–6]. The aim of this study was to validate the performance of an assay based on particle-enhanced turbidimetry, in order to measure CysC in human urine on a fully automated clinical chemistry analyzer.

Materials and methods

This study was approved by the hospital's scientific and ethical committee. Samples were collected from the routine at the clinical biochemistry department and were used anonymously. Spot urine collections (first morning void) were used in all measurements. Samples were collected in plain urine collection cups with no preservative, transported immediately to the lab, centrifuged at 2400 g for 5 min and analyzed within 1 h from collection. uCysC measurements were performed on an Abbott-architect ci16200 biochemistry analyzer (Abbott Laboratories, USA). For this purpose we used commercially available reagents (Sentinel, Italy). This method was originally designed and is validated for serum CysC measurements and is based on the latex particle-enhanced turbidimetric immunoassay (PETIA) principle. We used the same assay protocol as in the serum, modified only in calibration in order to cover lower values. A spline calibration method is used covering the range 0.05 to 2.58 mg/L. The total turnaround time of the assay is 10 min.

Abbreviations: (CysC), cystatin C; (uCysC), urinary cystatin C; (AKI), acute kidney injury; (PETIA), particle-enhanced turbidimetric immunoassay.

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0009-9120/$ – see front matter
http://dx.doi.org/10.1016/j.clinbiochem.2013.05.072

The assay's precision was determined by calculating the CVs (within-run, between-run and total CV) for 4 urine samples with concentrations of 0.20, 0.42, 1.25 and 2.10 mg/L for 20 consecutive days. A urine sample with high (2.25 mg/L) and a sample with very low concentrations of CysC (0.02 mg/L) were used in order to test linearity. A series of 10 dilutions was prepared by mixing different portions of the high and the low concentration samples. The recovery was tested by serially diluting a sample of initial value 10.6 mg/L. A urine sample with a concentration of 0.21 mg/L was used to spike the diluted samples. The linearity and recovery measurements were performed in triplicate. The limit of quantitation (LoQ) was tested by preparing serial dilutions of a sample with an initial value of 3.61 mg/L and the LoD was determined by measuring a sample with a zero concentration of CysC 20 times. Calibration stability was determined by calibrating the assay on the analyzer and running 3 controls daily for one month using Levey–Jennings graphs. Analyte stability was tested with 9 urine samples covering a wide range of uCysC concentrations (0.14–2.05 mg/L), divided into 2 portions, one stored at room temperature and the other at +4 °C. Samples that were stored at room temperature were analyzed at 2, 4, 8, 24 and 48 h, while those that were stored at +4 °C were analyzed daily for 5 days. The percentage change in concentration was calculated. For analyte stability no preservatives were used during the urine storage. No microbial contamination was observed in any of the samples. Urine pH in all samples was between 5.5 and 7.0. The clinical relevance of the assay was tested on several patients with different forms of kidney disease. For this reason we selected, using standard diagnostic criteria [7–9], 30 patients with confirmed intrinsic-AKI (tubular disease) [AKI_TD], 30 patients with stable chronic kidney disease [SCKD], 15 patients with prerenal-AKI [PRA], and 25 patients with normal kidney function [NKF]. To determine the reference range of this assay, 30 urine samples from healthy individuals were used (selected from a group of amateur athletes). Results were expressed as median and interquartile ranges (Table 1). Statistical analyses were performed using MedCalc statistical software.

Results

The obtained CV values (within run, between run and total) never exceeded 4% at any level (Table 2). The LoQ was determined in our experiment at 0.06 mg/L. All results showed a CV < 10%. The LoD was calculated at 0.02 mg/L. Recovery ranged from 101.62 to 106.49%. The assay showed very good linearity and the assay linearity equation was $y = -0.0034 + 1.0012x$ with a regression of $r^2 = 0.9998$ over the studied interval. Calibration proved to be stable for at least one month. The values of the controls used ranged between 0.22 and 0.85 mg/L. The CV% for each control level never exceeded 3%.

All samples stored at 4 °C resulted in loss of uCysC, after 5 days of storage, ranging from 1.85% to 14.29%. On the other hand the samples stored at room temperature gave varied results with both increasing and decreasing concentrations after 48 h of storage ranging from −9.09% to +21.43%. The sample that showed the highest decrease in concentration was the sample with lowest initial concentration (0.14 mg/L). There was no significant difference (paired *t*-test) between fresh samples and those measured up to 48 h at room temperature or at 4 °C and thus uCysC is considered stable at these temperatures over these time periods. However there was a significant drop ($p < 0.05$) in refrigerated samples after that time but the actual change in value was <0.18 mg/L.

In healthy controls uCysC values ranged from non-detectable (<0.02 mg/L) to 0.13 mg/L while in patients, median uCysC concentration was significantly higher in the AKI_TD patients ($p < 0.0001$, Kruskall–Wallis test) compared to the NKF, SCKD and PRA patients (Table 1).

Discussion

A number of studies have evaluated the potential use of measuring uCysC as a marker of tubular damage. In healthy individuals CysC is excreted in very low concentrations by urine. This means that an increased amount of CysC in urine may indicate an injury of the tubular cells [8]. According to the upper reference value proposed by us (0.12 mg/L) only small concentrations of uCysc are present in the urine of healthy controls. This upper reference limit corresponds well with those previously published [2,10].

The use of uCysC as an early and sensitive biomarker of AKI is at a research stage and more studies should be performed before it can reliably be introduced and added to a panel of biomarkers for the evaluation of renal function. Several publications report good results of uCysC for the diagnosis of AKI and in monitoring tubular function in patients after kidney transplantation as well as in patients receiving nephrotoxic medication [3,4,11,12]. In our study we included only a small group of healthy controls together with patients with renal disorders that potentially cover a wide range of uCysC concentrations. Our data show that uCysC values are clearly elevated in the group of patients with confirmed tubular disease (Table 1). uCysC has the advantage of not being affected by inflammation or infection, is more stable in urine, with minimal biological variation and may have age and sex independent reference values (compared to other markers such as b2-microglobulin or a1-microglobulin) [13–15].

An accurate and robust method of quantitation of uCysC is necessary in order to use this assay for the diagnosis and the monitoring of patients with AKI. In this study we report the validation of a turbidimetric assay for the measurement of uCysC, using commercially available reagents, on a high throughput clinical chemistry analyzer. We demonstrated that the measurement of uCysC by PETIA is precise with small intra- and inter-assay variation. The assay precision was acceptable, as in all levels of used controls the CV's were low (<4%). Similar results were also shown in previous studies where the used method for measuring cystatin C was particle-enhanced nephelometric immunoassay (PENIA). Recent publications have shown minimal differences between PETIA and PENIA [5,10].

The LoQ (0.06 mg/L), is adequate and allows precise measurements of CysC at low concentrations. The recovery was within the acceptable limit of ±10% and based on these results, automatic dilutions of samples with concentration of CysC, over the highest standard provide acceptable results. The assay was linear up to 2.25 mg/L with a very good correlation and intercept, covering almost the entire calibration interval and the stability of calibration was excellent.

The stability of CysC in urine is acceptable and comparable with that of previous reports [10]. CysC is stable for many hours at room

Table 1
Urinary cystatin C values in healthy controls as well as in patients with, normal kidney function (NKF), pre-renal azotemia (PRA), stable chronic kidney disease (SCKD) and in patients with tubular disease (AKI_TD).

	NKF	PRA	SCKD	AKI_TD	Healthy controls	P
N	25	15	30	30	30	
Male/female	20/5	12/3	24/6	26/4	25/5	NS
Mean age (SD)	65.6 (8.8)	70.8 (8.2)	68.1 (8.0)	72.6 (8.5)	41.5 (7.9)	
Median uCysc (in mg/L)	0.05	0.15	0.13	1.47	0.04	P < 0.0001
10th percentile	0.03	0.08	0.05	0.27	0.02	
90th percentile	0.12	0.31	0.77	3.87	0.12	

Table 2
Analytical imprecision of uCysC assay. The mean concentration in mg/L and the CV of urinary cystatin C in four samples at different levels. Within run, between runs and total CV were calculated.

Concentration mg/L	0.20	0.42	1.25	2.10
n	20	20	20	20
Within run CV%	2.13	0.89	0.63	1.25
Between runs CV%	2.63	1.03	1.05	2.25
Total CV %	3.56	2.58	2.24	3.75

temperature allowing urine samples to be processed easily without any special requirements, at least when they are planned to be tested within the same day.

The uCysC turbidimetric assay is a rapid and easy method that can be used for fast measurements and with a short turnaround time. Our data indicate that uCysC can be processed on automated clinical chemistry analyzers. Preliminary data show that increased uCysC levels may indicate tubular dysfunction and allow its accurate detection among various types of kidney disease. Therefore its measurement could be easily added to the standard panel used to screen kidney pathologies even in emergency situations.

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