Renal extraction of cystatin C vs $^{125}$I-iothalamate in hypertensive patients

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Abstract

Background. Several markers are available to estimate the glomerular filtration rate (GFR) in patients. Cystatin C is a relatively new marker and has been suggested as an alternative for creatinine. Numerous studies have been performed to evaluate the usefulness of cystatin C to estimate GFR. The aim of this study is to compare the renal extraction of cystatin C with that of $^{125}$I-iothalamate in hypertensive patients.

Methods. Forty hypertensive patients with unilateral renal artery stenosis, and who used at least two antihypertensive agents, were studied. For the determination of the renal extraction ratio, blood samples were drawn simultaneously from the renal vein and the abdominal aorta. The renal extraction ratio was calculated as $\frac{([A] - [V])/[A]}{[V]}$, in which A is the plasma concentration of the compound from the abdominal aorta, and V is the plasma concentration of the compound from the renal vein.

Results. The mean difference between the renal extraction ratio of cystatin C and that of $^{125}$I-iothalamate was 0.002. The 95% confidence interval (CI) for the mean difference was $-0.036$ to $0.032$, which was not statistically significant. However, the limits of agreement were large ($-0.271$ and $0.267$).

Conclusions. Despite a lower reported glomerular sieving coefficient of cystatin C, the mean renal extraction of cystatin C was equal to the mean renal extraction of $^{125}$I-iothalamate in hypertensive patients, suggesting tubular secretion of cystatin C. Combined with the large variation in the renal extraction of cystatin C, these findings cast doubts on its usefulness as a glomerular filtration marker.

Keywords: cystatin C; GFR; $^{125}$I-iothalamate; renal extraction

Introduction

The ability to measure renal function accurately is essential for evaluating patients with suspected renal disease and for studying changes in renal function. Several markers are available to estimate the glomerular filtration rate (GFR), but creatinine is most commonly used. It is a metabolic product of creatine and phosphocreatine in the muscle and its production is proportional to the total muscle mass. This leads to a variation in serum creatinine concentration across age, gender, race, nutritional status and body composition that is independent from changes in GFR. Moreover, creatinine is not only filtered by the glomerulus, but also secreted by the proximal tubule. As a consequence, based on the plasma creatinine concentration, GFR is overestimated, particularly at lower GFR. As a result, studies with alternative markers for the estimation of GFR have been performed. Cystatin C is a relatively new marker and has been suggested as an alternative for creatinine [1,2]. Cystatin C is a proteinase inhibitor with a molecular weight of 13.3 kDa and is produced by all nucleated cells. It is freely filtered by the glomerular membrane, reabsorbed and completely metabolized in the proximal tubule and does not return to the circulation [3]. Several studies have been performed to evaluate the usefulness of cystatin C to estimate GFR [3–7]. Cystatin C is metabolized in the tubule and it is not possible to determine the urinary clearance of cystatin C. However, the renal extraction ratio is a parameter for renal function and expresses the glomerular filtration and tubular handling (tubular secretion and/or reabsorption) of a substance. Therefore, we compared the renal extraction ratio of cystatin C with the extraction...
ratios of creatinine and $^{125}$I-iothalamate in order to determine the usefulness of cystatin C as an estimate of GFR.

Subjects and methods

Patients

We studied 40 consecutive patients with suspected unilateral renal artery stenosis. All patients had a diastolic blood pressure higher than 95 mmHg and used at least two antihypertensives. The study was approved by the hospital review board and done during the diagnostic work-up for renal artery stenosis. Renal function was measured using the constant infusion clearance technique of $^{125}$I-iothalamate without urine collections [8]. For the determination of the renal extraction ratio, blood samples were drawn simultaneously from the renal vein and the abdominal aorta in sodium citrate tubes; first at one side and immediately thereafter on the other. Before each blood sampling the correct positioning of the catheter in the renal vein was confirmed by X-ray control and oxygen saturation measurement.

The renal extraction ratio of cystatin C (Ecyst), creatinine (Ecreat) and $^{125}$I-Iothalamate (Ethal) were calculated as $([A] - [V])/[A]$, in which A is the plasma concentration of the compound from the abdominal aorta, and V is the plasma concentration of the compound from the renal vein.

Analysis

Plasma samples were stored at $-20\,^\circ\text{C}$ until analysis. Cystatin C was measured in triplicate using a fully automated particle enhanced immuno turbidimetric method (DAKO Cystatin C PET kit, Copenhagen, Denmark). The assay was performed on a Hitachi 912 auto-analyzer (Roche Diagnostics, Basel, Switzerland). Inter- and intra-assay variation, calculated from the control samples with assigned values of 1.4 and 2.8 mg/l, were 11.3 and 5.6%, respectively. Triglyceride concentration was measured in all samples, as a concentration of triglycerides $>4$ mmol/l interferes with the assay for cystatin C. The plasma creatinine concentration was measured enzymatically on a Hitachi 912 (Roche Diagnostics, coefficient of variation 1.7 and 2.7% for a concentration of 80 and 400 $\mu$mol/l, respectively).

Statistical analysis

Data are presented as mean $\pm$ SD. Agreement between Ecyst C and Ethal, and Ecreat and Ethal was evaluated as described by Bland and Altman [9]. The limits of agreement represent the range around the mean difference between both methods in which 95% of the values will be found (mean difference $\pm 1.96$ SD). The 95% Confidence intervals (CIs) of the mean difference were calculated by the mean difference $\pm t\times SE$ of the mean difference, in which $t$ is the appropriate point of the $t$-distribution with $n-1$ degrees of freedom. The statistical analysis was made by using GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, USA).

Results

Forty hypertensive patients with unilateral renal artery stenosis were studied (male/female: 21/19; mean age: 55 $\pm$ 13 years). All plasma samples had a triglyceride concentration lower than 4 mmol/l.

The mean plasma clearance of $^{125}$I-iothalamate was 61 $\pm$ 21 ml/min/1.73 m$^2$ and ranged from 27 to 108 ml/min/1.73 m$^2$. For one patient, the plasma clearance of $^{125}$I-iothalamate was not available. There was a significant positive correlation between the reciprocal cystatin C concentration and the plasma clearance of $^{125}$I-iothalamate (Figure 1). The squared correlation coefficient was 0.538.

In total, 80 samples of the left and right kidney from 40 patients were available to determine the renal extraction ratio of cystatin C. In 16 cases, the sample volume was insufficient for analysis in triplicate. The range of renal extraction ratios varied from 0.01 to 0.54 for cystatin C and 0.01 to 0.33 for $^{125}$I-Iothalamate (Table 1). The mean difference between Ecyst C and Ethal was small (0.002), however the limits of agreement for the mean difference were large ($-0.271$ and 0.267) (Figure 2A). The 95% CI for the mean difference was $-0.036$ to 0.032, which shows that the mean difference was not statistically significant.

From the first 20 patients the extraction ratio of creatinine was also available (36 samples). The mean difference between the renal extraction ratio of creatinine and $^{125}$I-Iothalamate was 0.014 with a 95% CI of $-0.011$ to 0.039 (limits of agreement: $-0.131$ and 0.159) (Figure 2B).

Discussion

This study represents the first description of direct measurement of renal extraction of cystatin C. We observed that the mean difference between the extraction ratios of cystatin C and $^{125}$I-Iothalamate was...
not statistically significant. However, the limits of agreement showed a large range. In general, the renal extraction ratio shows a larger variation than the GFR, since a small variation in concentration has a large impact on the value of the extraction ratio. However, the cystatin C concentration was measured in triplicate, so it seems that this argument could not fully explain the observed variation.

The large limits of agreement (i.e. variation in the renal extraction of cystatin C) suggest that the theory that cystatin C is readily filtered in the glomerulus (sieving coefficient of 1) and subsequently metabolized in the tubule is untrue. Given its molecular weight of 13.3 kDa, which is greater than that of either creatinine (0.113 kDa) or inulin (5.2 kDa), the sieving coefficient of cystatin C may not equal 1. Tenstad et al. reported a sieving coefficient for cystatin C between 85 and 94% [10]. As a consequence, the extraction ratio of cystatin C should be a little bit smaller than that of $^{125}\text{I}-\text{iothalamate}$, but we found no difference between the renal extraction ratio of cystatin C and $^{125}\text{I}-\text{iothalamate}$, suggesting that cystatin C also undergoes tubular secretion.

The correlation coefficient for the relationship between the reciprocal cystatin C concentration and the plasma clearance of $^{125}\text{I}-\text{iothalamate}$ was 0.73 ($R^2 = 0.538$), which is comparable with earlier reported correlation coefficients. Coll et al. mentioned a correlation coefficient of 0.77 for the relationship between the reciprocal cystatin C concentration and the plasma clearance of $^{125}\text{I}-\text{iothalamate}$ in 61 patients [11]. Risch et al. found a correlation coefficient of 0.83 for the relationship between the reciprocal cystatin C concentration and the urinary clearance of $^{125}\text{I}-\text{iothalamate}$ in 30 patients [12].

In this study, $^{125}\text{I}-\text{iothalamate}$ was used as reference marker. For creating Bland–Altman plots it is important that the reference method is suitable, otherwise the conclusion can be incorrect. For the mean difference of renal extraction ratio of cystatin C and $^{125}\text{I}-\text{iothalamate}$ a large variation was observed (Figure 2A). If the renal

| Number | Left kidney | Right kidney |
|--------|-------------|--------------|
|        | Ecyst C | Ethal | Ecyst C | Ethal |
| 1      | 0.15    | 0.12 | 0.17    | 0.21 |
| 2      | 0.03    | 0.08 | na      | na  |
| 3      | 0.16    | 0.27 | 0.06    | 0.11 |
| 4      | 0.09    | 0.05 | 0.27    | 0.28 |
| 5      | 0.25    | 0.13 | 0.12    | 0.19 |
| 6      | 0.30    | 0.05 | 0.24    | 0.18 |
| 7      | 0.24    | 0.12 | 0.17    | 0.23 |
| 8      | 0.19    | 0.25 | na      | na  |
| 9      | 0.21    | 0.08 | 0.15    | 0.26 |
| 10     | 0.32    | 0.15 | 0.11    | 0.24 |
| 11     | 0.24    | 0.04 | 0.25    | 0.20 |
| 12     | na      | na   | 0.14    | 0.31 |
| 13     | 0.11    | 0.22 | 0.10    | 0.26 |
| 14     | 0.04    | 0.22 | 0.02    | 0.11 |
| 15     | 0.01    | 0.01 | 0.24    | 0.33 |
| 16     | 0.16    | 0.21 | 0.20    | 0.14 |
| 17     | 0.24    | 0.18 | 0.13    | 0.19 |
| 18     | na      | na   | 0.26    | 0.27 |
| 19     | na      | na   | 0.15    | 0.14 |
| 20     | 0.16    | 0.14 | 0.10    | 0.25 |
| 21     | 0.05    | 0.10 | 0.13    | 0.21 |
| 22     | na      | na   | 0.21    | 0.14 |
| 23     | na      | na   | 0.24    | 0.14 |
| 24     | na      | na   | 0.10    | 0.24 |
| 25     | 0.42    | 0.09 | na      | na  |
| 26     | 0.01    | 0.15 | 0.18    | 0.24 |
| 27     | 0.10    | 0.20 | 0.42    | 0.20 |
| 28     | 0.33    | 0.16 | 0.07    | 0.20 |
| 29     | na      | na   | 0.23    | 0.29 |
| 30     | na      | na   | 0.42    | 0.21 |
| 31     | 0.08    | 0.22 | na      | na  |
| 32     | 0.08    | 0.23 | 0.16    | 0.22 |
| 33     | 0.54    | 0.27 | 0.43    | 0.21 |
| 34     | na      | na   | 0.39    | 0.19 |
| 35     | 0.24    | 0.33 | 0.23    | 0.27 |
| 36     | na      | na   | 0.08    | 0.31 |
| 37     | na      | na   | 0.08    | 0.14 |
| 38     | 0.02    | 0.25 | na      | na  |
| 39     | 0.15    | 0.13 | 0.27    | 0.15 |
| 40     | 0.44    | 0.13 | 0.03    | 0.18 |

na = not available.
extraction of $^{125}$I-iothalamate had a large variation, a large variation was also found for the mean difference of Ecreat and Ethal (Figure 2B). Since that variation was smaller, the use of $^{125}$I-iothalamate as reference marker cannot explain the large variation found.

The present study was performed in hypertensive patients who used two or more antihypertensive agents. It is not clear whether similar results will be found in non-hypertensive patients, since it is theoretically possible that antihypertensive agents (such as angiotensin converting enzyme inhibitors or angiotensin II antagonists) change the renal extraction of cystatin C. However, we compared the renal extraction ratio of cystatin C with that of $^{125}$I-iothalamate and the effect of the antihypertensive agents in glomerular filtration would equally affect both markers. Moreover, Knight et al. studied the effect of several factors on the serum cystatin C concentration and found that the presence of hypertension was not significantly associated with an increase in cystatin C concentration [13]. Therefore, the results can be extrapolated to other patient populations.

We conclude that despite a lower reported glomerular sieving coefficient of cystatin C, the mean renal extraction of cystatin C was equal to the mean renal extraction of $^{125}$I-iothalamate in hypertensive patients, suggesting tubular secretion of cystatin C. Combined with the large variation in the renal extraction of cystatin C, these findings cast doubts on its usefulness as a glomerular filtration marker.

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