

# Serial Measurements of Cystatin C Are More Accurate Than Creatinine-Based Methods in Detecting Declining Renal Function in Type 1 Diabetes

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**OBJECTIVE**— Cystatin C- and creatinine-based methods were compared with <sup>99m</sup>Tc-technetium-diethylene-triamine-penta-acetic acid (<sup>99m</sup>Tc-DTPA) plasma clearance (isotopic glomerular filtration rate [iGFR]) for detecting declining renal function.

**RESEARCH DESIGN AND METHODS**— Glomerular filtration rate (GFR) was monitored over a mean of 10.1 years in 85 subjects with type 1 diabetes (with an average of 5.6 measurements per individual). Baseline mean  $\pm$  SD iGFR of the cohort was  $106.1 \pm 2.6$  ml/min per  $1.73 \text{ m}^2$ . The rates of decline in GFR ( $\Delta$ GFR) were derived using linear regression.

**RESULTS**— In 19 of 85 subjects with declining renal function (i.e.,  $\Delta$ iGFR  $>3.3$  ml/min per  $1.73 \text{ m}^2$  per year),  $\Delta$ GFR (ml/min per  $1.73 \text{ m}^2$  per year) was 6.5 by iGFR, 4.2 by  $10^4$ /creatinine, 3.6 by Cockcroft-Gault formula, 3.4 by the Modification of Diet in Renal Disease (MDRD)-6 equation, and 3.5 by the MDRD-4 variable equation ( $P < 0.01$  vs. iGFR). In comparison,  $\Delta$ GFR was 6.1 using the formula  $\text{Cys-GFR} = (86.7/\text{cystatin C concentration}) - 4.2$  (not significant).

**CONCLUSIONS**— Cystatin C was more accurate in detecting decline in renal function than creatinine-based methods in this population of subjects with type 1 diabetes and a normal mean baseline GFR.

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**M**onitoring trends in renal function via glomerular filtration rate (GFR) is of critical importance in managing patients with diabetes. Gold-standard methods for determining GFR employing plasma clearance techniques are cumbersome and not adaptable to routine clinical practice. Limitations of serum creatinine- and creatinine-based equations for estimation of GFR are well known (1,2). Cystatin C concentration has been proposed as an endogenous marker of GFR superior to creatinine (3,4).

We compared serum cystatin C, creatinine, Cockcroft-Gault formula, and the Modification of Diet in Renal Disease

(MDRD)-4 and -6-variable equations with direct measurement of renal function employing <sup>99m</sup>Tc-technetium-diethylene-triamine-penta-acetic acid (<sup>99m</sup>Tc-DTPA) plasma clearance (isotopic GFR [iGFR]) in longitudinal monitoring of GFR in 85 subjects with type 1 diabetes with baseline GFR values predominantly in the normal range. In particular, the accuracy of the above methods for identifying subjects with progressively declining renal function was examined.

## RESEARCH DESIGN AND METHODS

A search of our database located 85 subjects with type 1 diabetes attending the Diabetes Clinics at

Austin Health (a tertiary referral center) in Melbourne, Australia, in whom at least two iGFR measurements had been performed 3 or more years apart between 1987 and 2004. Subjects with nondiabetic renal disease were excluded. All subjects were Caucasian, except for one who was of Chinese ethnicity.

GFR measurements utilizing <sup>99m</sup>Tc-DTPA plasma clearance (iGFR) were routinely performed (5,6) on all clinic attendees two to three times yearly, irrespective of renal function or albuminuria status.

At baseline, mean  $\pm$  SEM age of the 85 subjects ( $n = 85$ , 49 male) was  $38.4 \pm 1.3$  years (range 14–72) and the mean  $\pm$  SEM disease duration was  $13.7 \pm 1.1$  years (range 0.2–48.6). On average, 5.6 iGFR measurements had been performed on each individual (range 2–11). The initial mean  $\pm$  SEM iGFR was  $106.1 \pm 2.6$  ml/min per  $1.73 \text{ m}^2$ , which declined to  $90.4 \pm 3.4$  ml/min per  $1.73 \text{ m}^2$  after  $10.1 \pm 0.3$  years (range 3.0–15.7) of follow-up. The initial iGFR was  $>120$  in 27%, 90–120 in 54%, and  $<90$  ml/min per  $1.73 \text{ m}^2$  in 19% of the subjects. From these 85 subjects, those with a rate of decline in iGFR  $>3.3$  ml/min per  $1.73 \text{ m}^2$  per year were defined as having a declining renal function (“decliners,”  $n = 19$ ) based on longitudinal data from the Baltimore Longitudinal Study of Aging (7).

Serum for creatinine and cystatin C was collected on the same morning as the corresponding iGFR measurement. Creatinine assays were determined by the Jaffe alkaline picrate method in the same laboratory, using a Parallel American Monitor (1987–1993), a Hitachi 747 instrument (1994–1997), or a Hitachi 917 automatic analyzer (1998–2004). There was no difference between GFR estimates obtained with the three creatinine methods and the corresponding iGFR measurements when analyzed by the Bland-Altman method. The latter assay produced creatinine values that fell within  $\pm 15\%$  of the reference MDRD method, an accuracy that has been en-

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**Abbreviations:** GFR, glomerular filtration rate; iGFR, isotopic GFR; MDRD, Modification of Diet in Renal Disease; <sup>99m</sup>Tc-DTPA, <sup>99m</sup>technetium-diethylene-triamine-penta-acetic acid.

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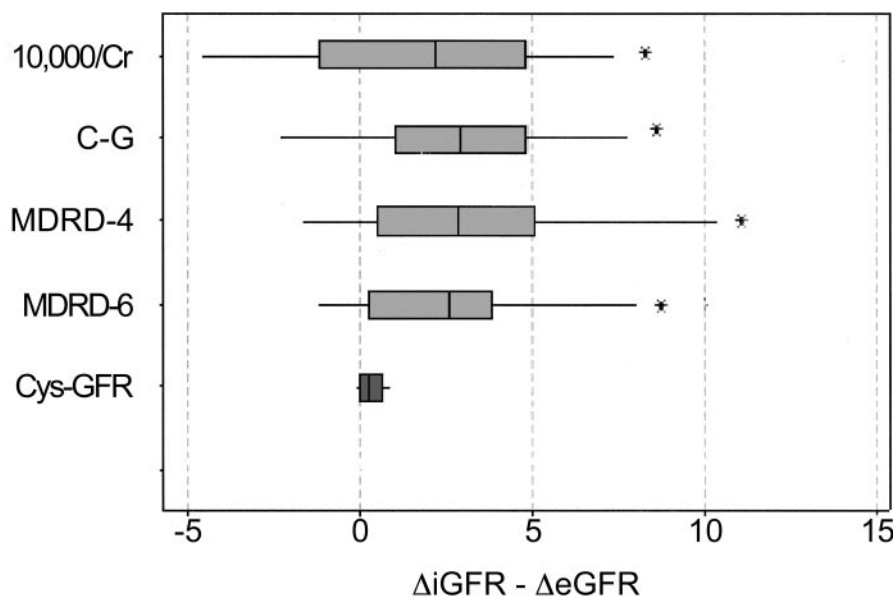
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dorsed by the Australian Creatinine Consensus Working Group on automatic reporting of estimated GFR (8). The intra- and interassay coefficients of variation (CVs) for serum creatinine were 2.3 and 6.7%, respectively, using the Hitachi 917 analyzer. Cystatin C was measured from stored serum samples in a single batch in 2004 using an automated particle-enhancing immunonephelometric assay on a BN II instrument (Dade Behring, Marburg, Germany). The intra- and inter-assay CVs for cystatin C were 2.58 and 3.95%, respectively, at a concentration of 1.54 mg/l.

The following techniques were used to estimate GFR: creatinine was transformed to a GFR value (Cr-GFR) using the equation  $10^4/\text{serum creatinine}$  ( $\mu\text{mol/l}$ ). Cystatin C was transformed to a GFR equivalent using the equation  $\text{Cys-GFR} = (86.7/\text{cystatin C concentration}) - 4.2$  (3). Standard formulas were used to derive GFR from Cockcroft-Gault formula (CG-GFR) (9) and MDRD equations (1). For each individual, the trends of GFR ( $\Delta\text{GFR}$ ) were derived from linear regression from each method. The regression line for all except one subject with declining GFR was statistically significant ( $P < 0.05$ ). Statistical analysis was performed using Minitab 14 statistical software.

**RESULTS**— The baseline and final iGFR in the group of decliners ( $n = 19$ ) was mean  $\pm$  SEM  $105.1 \pm 7$  and  $51.6 \pm 7$  ml/min per  $1.73 \text{ m}^2$ , respectively, compared with  $106.3 \pm 3$  and  $101.5 \pm 3$  ml/min per  $1.73 \text{ m}^2$  in the nondecliners. Decliners had a greater baseline A1C than nondecliners ( $P < 0.05$ ). The median baseline and final albumin excretion rates were 18 and 150  $\mu\text{g}/\text{min}$ , respectively, in decliners and 9 and 10  $\mu\text{g}/\text{min}$ , respectively, in nondecliners. Thus, both baseline and final albumin excretion rate was higher in decliners than nondecliners ( $P < 0.02$ ).

The mean  $\Delta\text{iGFR}$  in the 19 decliners was 6.5 (range 3.3–26.2) ml/min per  $1.73 \text{ m}^2$  per year. All of the creatinine-based methods significantly underestimated the decline in iGFR, i.e., 4.2 for Cr-GFR ( $P < 0.01$  vs.  $\Delta\text{iGFR}$ ), 3.6 for CG-GFR ( $P < 0.01$ ), 3.4 for MDRD-4-GFR ( $P < 0.01$ ), and 3.5 for MDRD-6-GFR ( $P < 0.01$ ). In contrast, there was no difference between rates of decline observed with iGFR and those observed with cystatin C, i.e.,  $\Delta\text{Cys-GFR}$  6.1 ml/min per  $1.73 \text{ m}^2$  per year (Fig. 1). The sensitivity and specificity for each of the four creatinine-based



**Figure 1**— Comparison of the difference between the rates of decline in GFR (ml/min per  $1.73 \text{ m}^2$  per year) as measured by  $^{99\text{m}}\text{Tc}$ -DTPA clearance ( $\Delta\text{iGFR}$ ) and the various indirect estimates of GFR ( $\Delta\text{eGFR}$ ) in “decliners,” i.e., subjects with a  $\Delta\text{iGFR} > 3.3$  ml/min per  $1.73 \text{ m}^2$  per year ( $n = 19$ ). Cr-GFR,  $10^4/\text{serum creatinine}$ ; C-G, Cockcroft-Gault formula; Cys-GFR,  $(86.7/\text{serum cystatin C concentration}) - 4.2$  (3). The line in the box represents the median and the boxes represent the interquartile range. Asterisks indicate whether ( $\Delta\text{iGFR} - \Delta\text{eGFR}$ ) is significant ( $P < 0.05$ ).

methods for identifying the 19 subjects with declining iGFR were 42 and 100%, respectively, compared with 84 and 100%, respectively, for Cys-GFR.

**CONCLUSIONS**— This study demonstrates that estimates of renal function derived from serum cystatin C accurately portray long-term changes in GFR when compared with serial iGFR measurements in a cohort of subjects with type 1 diabetes with declining renal function. In contrast, creatinine-based estimates such as the Cockcroft-Gault formula and MDRD-4 and -6 variable equations and  $1/\text{creatinine}$  significantly underestimated the decline in iGFR in this population.

Thus serial measurements of serum cystatin C may facilitate early identification of patients at risk of developing renal failure. Cystatin C has the potential to be employed more widely for monitoring of GFR, given its advantages of availability of a simple and accurate automated assay and the ability to directly use the reciprocal of cystatin C level as a GFR equivalent.

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