Mini-Review

Estimating Glomerular Filtration Rate in Diabetes Using Serum Cystatin C

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Abstract
Chronic kidney disease (CKD) is a major public health problem, especially for people with diabetes. Not only is it a risk factor for end-stage renal disease (ESRD) but it is also a major cardiovascular disease (CVD) risk factor. Methods that accurately and simply estimate glomerular filtration rate (GFR) are therefore needed to optimise the detection and management of CKD in people with diabetes. One of the main failures of commonly used creatinine-based methods for estimating renal function is that they lack applicability across the full range of GFR values and underestimate GFR levels >60 mL/min/1.73m^2. Methods for accurately estimating an early pathological decline in GFR (i.e. ΔGFR >3.3 mL/min/year before reaching a GFR <60 mL/min/1.73m^2) are especially needed as appropriate interventions have been shown to retard progression to ESRD and reduce CVD risk in people with diabetes. In contrast, recent studies have suggested that estimates of GFR based on serum cystatin C concentration might provide a simple and accurate method for detecting and monitoring an early decline in renal function.

Introduction

Diabetes is recognised as a major risk factor for the development of CKD. In developed countries, diabetes is the leading cause of ESRD and diabetic subjects with CKD are at high risk for developing CVD. GFR is usually accepted as the best overall estimate of kidney function and therefore is commonly used to evaluate onset and progression of CKD. Currently, the most common method for estimating GFR is based on serum creatinine concentration and uses the four-variable Modification of Diet in Renal Disease (MDRD) equation. Unfortunately, this approach has been well documented to significantly underestimate GFR levels in the high-normal range and therefore lacks sufficient sensitivity to detect early, but pathological declines in GFR. The early detection of kidney dysfunction in subjects with diabetes is of vital importance as appropriate interventions have been shown to retard the progression to ESRD and reduce CVD. Recently there has been considerable interest in cystatin C as a surrogate marker for estimating GFR, especially in its potential to accurately estimate an early GFR decline in individuals with diabetes.

Inadequacies of Creatinine-Based Methods for Estimating GFR

Current gold standard methods for determining GFR, employing the clearance of exogenous radioisotopes or non-radiolabelled markers, are time-consuming and expensive, and are thus not easily adaptable to routine clinical practice. Therefore, creatinine has been used as an endogenous marker of GFR for many decades. Unfortunately, the influence of non-renal factors on serum creatinine concentration, including age, gender, ethnicity, muscle mass and dietary protein intake, limit its usefulness as a marker of GFR. To overcome the limitations of using creatinine alone, equations to estimate GFR (eGFR) based on serum creatinine have been developed that include variables such as age, sex, race and measurements of body size.[1]

Prior to the introduction of the MDRD equation, the Cockcroft-Gault (C-G) formula was the most widely used equation to estimate GFR. This formula provides an estimate of creatinine clearance and not true GFR. Furthermore, it usually overestimates GFR in people with reduced kidney function because of the tubular secretion of creatinine. Another limitation of the C-G formula is that it estimates renal function in proportion to weight and is not routinely normalised for body surface area. This is of special importance in subjects with type 2 diabetes as most are overweight or obese.[2]

The MDRD formula incorporates information about age, gender, ethnicity and serum creatinine concentration, and was developed from a population of predominantly non-diabetic

subjects with a decreased GFR (mean GFR approximately 40 mL/min/1.73m^2).[3] The formula was derived from a reference GFR measurement using an iothalamate clearance technique. The MDRD formula has generally been reported to outperform the C-G formula for reference GFR levels <60 mL/min/1.73m^2. Its use in the automatic reporting of eGFR by laboratories is facilitated by its lack of reliance on incorporation of weight into the formula. Since the publication of the original four-variable MDRD formula, a '175' formula has also been developed which uses the same variables as in the original formula but is based on creatinine measurements traceable to an isotopic dilution mass spectrometry reference method. This '175' MDRD formula is currently the method recommended for the automatic reporting of eGFR by clinical chemistry laboratories in Australia.[4]

Recently, studies have highlighted the limitations of existing creatinine-based methods for estimating renal function in diabetes. In summary, for patients with a GFR in the normal or hyperfiltering range, GFR is grossly underestimated by the MDRD formula by approximately 10 to 40%. Most studies have shown that GFR levels within this range are also underestimated by the C-G formula but some have reported an overestimation. The wide variation in estimated renal function by the C-G formula most likely relates to the characteristics of the different populations studied, especially differences in body weight, muscle mass and whether a correction for body surface area was used. In addition, longitudinal studies demonstrated that both the MDRD and C-G formulae also significantly underestimate the rate of decline in GFR when measured by a reference method as discussed later.[5,6]

To overcome the limitations of the MDRD equation, a new creatinine-based formula that incorporates the original variables used in the MDRD formula, has been devised to improve estimation of GFR in individuals with a GFR >60 mL/min/1.73m^2. The Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation was developed using data from 8254 individuals from 10 studies and validated in 3896 individuals from 16 studies.[7] The mean reference GFR in the population used to derive the CKD-EPI formula was 68 mL/min/1.73m^2. One recent study has shown that for reference GFR levels in the range 90–119 mL/min/1.73m^2, bias was reduced from 10.0 mL/min/1.73m^2 when GFR was estimated by the MDRD formula to only 1.9 mL/min/1.73m^2 when GFR was estimated by the CKD-EPI formula.[8] However, it should be noted that the CKD-EPI equation has yet to be rigorously tested in subjects with diabetes with high-normal GFR levels.

Cystatin C – A Potential Surrogate Marker of GFR

An alternative approach to currently used methods of estimating GFR is to use a different analyte to creatinine for developing GFR predicting equations. More than 20 years ago, Grubb proposed that cystatin C could be used as a potential marker of GFR based on the fact that, unlike creatinine, cystatin C levels are generally not influenced by extra-renal factors such as age, gender, diet and muscle mass.[9] Cystatin C is a low molecular weight (13.3 kDa) protein that is produced at a constant rate by all nucleated cells, freely filtered by the glomerulus and then completely metabolised by the proximal tubule. However, it is only recently that studies have supported the measurement of serum cystatin C as a simple, reliable and accurate marker of GFR. Automated assays for measuring cystatin C have been developed and an immunoassay based on particle-enhanced immunonephelometry is now the most common method used.[10] Over the last decade there have been multiple publications supporting the potential usefulness of estimating GFR using cystatin C-based methods. Although cystatin C is well established as a research tool it has yet to make the transition to use in routine clinical practice.

Do Factors apart from GFR Influence Serum Cystatin C Concentration?

Although cystatin C was originally reported to be free of non-renal influences, several extra-renal factors have recently been shown to modulate serum cystatin C levels. These include age, body weight, gender, smoking, C-reactive protein, abnormal thyroid status, malignant and inflammatory states, and steroid therapy. Indeed, studies involving the use of reference, non-creatinine based GFR measurements have suggested that factors other than GFR do influence cystatin C levels. Furthermore, there is evidence to suggest that compared with a reference GFR (iothalamate clearance) measurement, cystatin C is a better predictor of cardiovascular events and total mortality. This suggests that cystatin C concentrations could reflect some other deleterious process apart from reduced kidney function.[11]

Even if these limitations are proven to be correct, evidence continues to accumulate suggesting that cystatin C methods for estimating GFR, without correction for any anthropometric or biochemical variables, are equal or superior to creatinine-based methods in subjects with and without diabetes.

We have investigated the independent clinical and biochemical associations with serum cystatin C concentration and reference isotopic-diethylenetriaminepenta-acetic acid (DTPA) GFR levels in subjects with type 2 diabetes. In our study population, the same five variables, i.e. age, urinary albumin excretion rates, haemoglobin, history of macrovascular disease and triglyceride concentration were independently associated with both cystatin C and reference GFR levels.[12] No associations with measurements of body size or markers of inflammation were found, contrary to the

results of some other studies. The relationship between age and serum cystatin C levels is shown in Figure 1. We found that when subjects were first stratified according to different levels of GFR and then subsequently into different age groups, at any given level of GFR, regardless of age, subjects had similar serum cystatin C concentrations. This indicates that the main reason why cystatin C concentrations have been reported to increase with age is because of the expected age -related decrease in GFR.

The Usefulness of Cystatin C in Estimating Various Levels of GFR

For subjects with mild to moderate CKD, most studies have shown that cystatin C is equal or superior to creatinine-based methods for estimating a reference GFR measurement. Our own studies[12] have shown that for detection of renal impairment (reference isotopic-GFR levels of <60 mL/min/1.73m^2) in subjects with diabetes, serum cystatin C concentrations alone are at least as sensitive and specific as creatinine-based methods including the four-variable MDRD equation, as shown in Figure 2. Moreover, at high-normal reference GFR levels, estimates of GFR based on cystatin C have clearly been shown to outperform those based on the MDRD and C-G formulae. Currently, there are no studies in subjects with diabetes comparing the performance of estimates of GFR based on cystatin C with those derived from the CKD-EPI formula.

Several formulae have been developed to estimate GFR based on the reciprocal of serum cystatin C levels. Generally, whatever cystatin C-based formula is used, bias is smaller and precision greater compared with the creatinine-based MDRD formula for estimating a reference GFR measurement. For example, in a study of approximately 100 subjects with diabetes and a mean reference–isotopic GFR measurement of 104 mL/min/1.73m^2, the MDRD formula underestimated the isotopic GFR level by 27 mL/min/1.73m^2 whereas GFR estimated from an equation developed by our group and based solely on serum cystatin C concentration (GFR = 88/cystatin C - 4.1) underestimated the reference GFR measurement by only 2.4 mL/min/1.73m^2.[12,13] Our cystatin C formula also had the greatest accuracy (smallest bias), precision (standard deviation of the bias) and proportion of estimated GFR measurements within 30% of isotopic GFR measurements compared with six other GFR equations based on serum cystatin C concentration that have been recently published (Table).

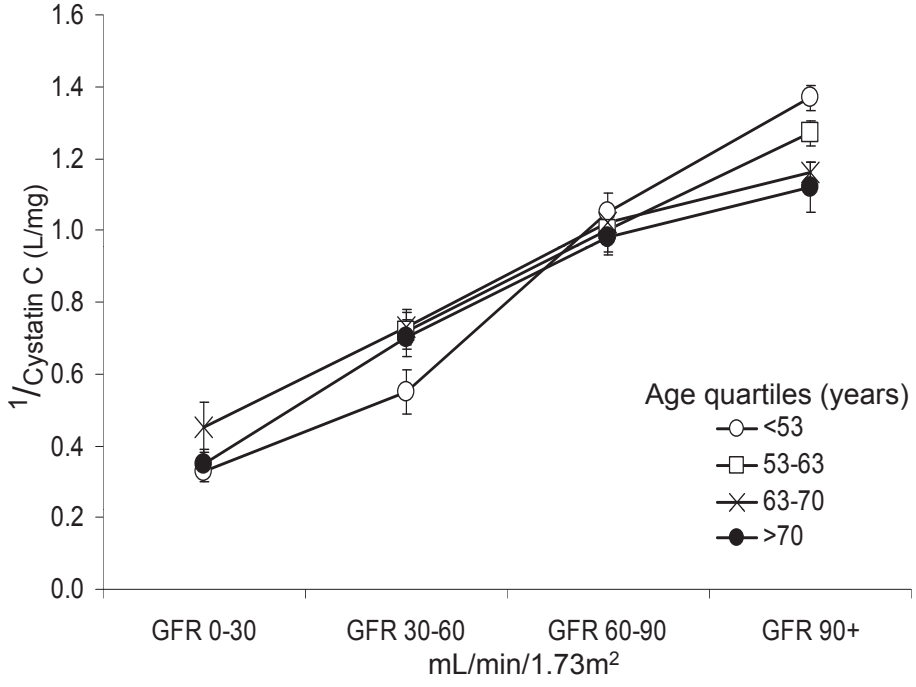

Figure 1. Age, GFR and cystatin C concentration. In this study GFR was measured by the plasma isotopic-DTPA clearance in 251 subjects with type 2 diabetes.

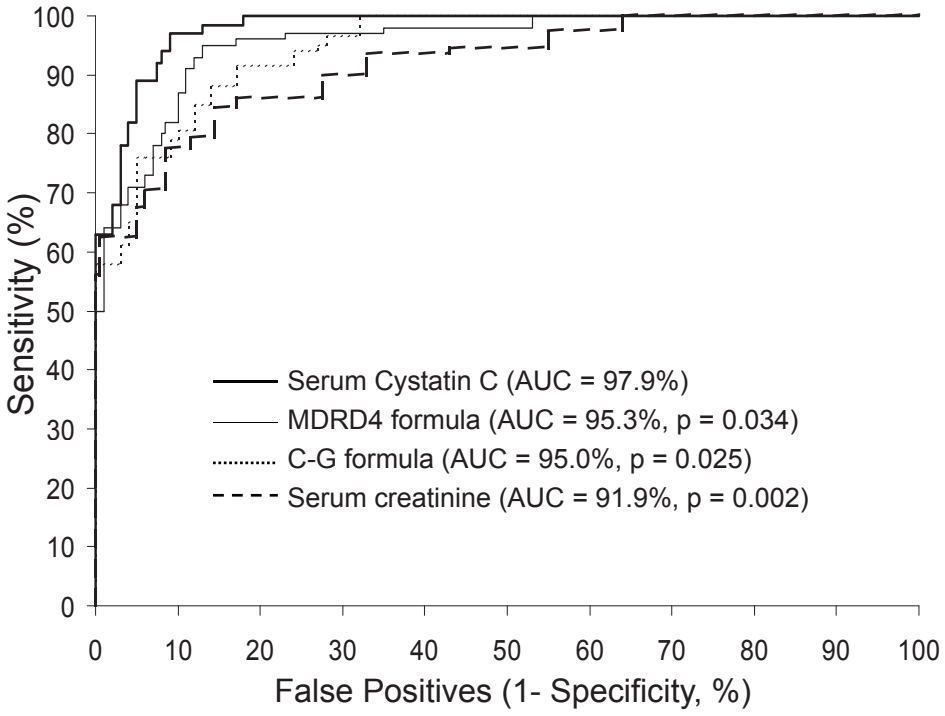

Figure 2. The accuracy of cystatin C and various creatinine-based methods of estimating GFR in determining an isotopic-DTPA GFR measurement <60 mL/min/1.73m^2.[12] The p values compare the area under the curve (AUC) for various creatinine-based methods for estimating GFR versus the AUC for the cystatin C method.

Table. Comparison of the performance of the MDRD and various cystatin C-based formulae for estimating GFR of more than 60 mL/min, adapted from the study by Chudleigh *et al.* with permission.[13] In this study of 100 subjects with diabetes, the mean reference isotopic GFR measurement ± SD was 104.5 ± 20 mL/min/1.73m^2. A full description of the formulae used in the study is contained in the above reference.[13] Bias is the mean difference between a reference isotopic GFR and GFR estimated by the various formulae. Precision is the standard deviation of the bias. Accuracy refers to the proportion of results that fall within 10% or 30% of reference isotopic GFR measurements.

	Reference-isotopic GFR 105 mL/min/1.73m^2				
Formula	**eGFR**	**Bias**	**Precision**	**Accuracy ,%**	
				10%	**30%**
MDRD	77.4	-27.1	18.0	10	65
Perkins	124.5	20.0	29.5	21	64
Arnal	101.7	-2.8	32.2	30	75
Rule	90.0	-14.5	28.4	31	68
MacIsaac	102.1	-2.4	26.0	34	85
Tan	101.6	-2.9	26.6	34	84
Stevens *	96.0	-8.5	28.1	29	75
Stevens †	85.6	-18.9	19.0	27	78

* Formula incorporates cystatin C, age and gender.
† Formula incorporates cystatin C, age and gender plus creatinine levels.

Furthermore, serum cystatin C concentrations have also been demonstrated to increase in a stepwise fashion in parallel with decreases in GFR for patients with diabetes.[14] This relationship is evident even when patients are divided into those with a GFR >120 and those with a GFR of 90 to 119 mL/min/1.73m^2 (Figure 3).

Assessing Longitudinal Changes in GFR with Cystatin C

In hyperfiltering (baseline GFR of 153 mL/min/1.73m^2) Pima Indian subjects with type 2 diabetes, trends in the reciprocal of serum cystatin C concentration have been shown to more closely reflect changes in GFR measured by iothalamate clearance than have creatinine-based estimates of renal function.[5] In that study, trends in GFR derived from the reciprocal of cystatin C (100/cystatin C) were closely correlated with an iothalamate-based reference method over a follow-up period of four years (r = 0.77), whereas correlation between the reference method and the C-G formula or the MDRD equation was not significant.

In patients with type 1 diabetes, we have estimated the rates of decline of GFR using creatinine-based formulae and compared these with rates of decline in GFR measured in plasma by a reference isotopic-DTPA disappearance technique and by estimated GFR derived solely from serum cystatin C concentration (See Figure 4 legend for details of formulae used to estimate GFR). In a cohort of 85 patients with an initial mean isotopic-DTPA GFR of 106 mL/min/1.73m^2, the measured reference rate of decline over 10 years was 1.8 mL/min/1.73m^2 per year, and the cystatin C-based estimate of rate of decline was 1.9 mL/min/1.73m^2 per year.[6] By contrast, creatinine-based estimates rates of GFR decline were only 0.7–0.8 mL/min/1.73m^2 per year, suggesting that creatinine-based estimates of GFR significantly underestimate the rate of progression of nephropathy. Furthermore, in a sub-study of 19 subjects with an initial isotopic-DTPA GFR of 105 mL/min/1.73m^2 and a rate of GFR decline >3.3 mL/min/1.73m^2 per year (mean isotopic-DTPA GFR rate of decline 6.5 mL/min/1.73m^2 per year), the rate of progression by creatinine-based formulae ranged from 3.5 to 4.1 mL/min/1.73m^2 per year. By contrast, the cystatin C-estimated rates of decline of GFR were significantly greater, between 6 and 7 mL/min/1.73m^2 per year, and close to the reference measure (Figure 4). Cystatin C-based estimates of changes in GFR are therefore more accurate than creatinine-based methods for estimating an early decline in GFR.

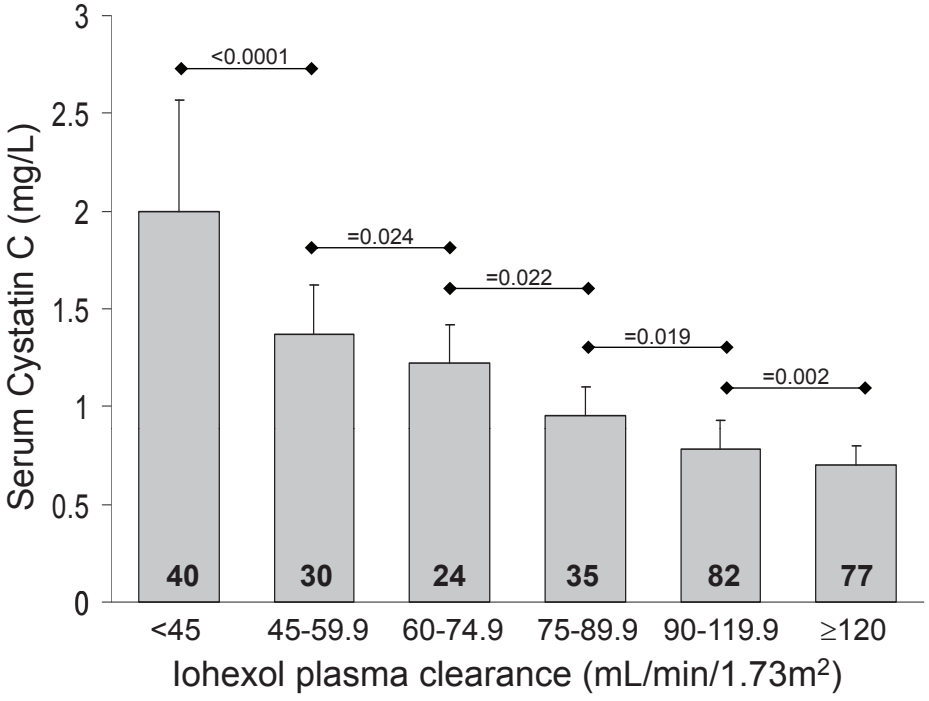

Figure 3. Serum cystatin C levels stratified according to different reference GFR levels. In this study GFR was assessed by iohexol plasma clearance in 288 patients with either type 1 or 2 diabetes. The numbers within each bar represent the number of subjects studied at that level of GFR. Adapted from Pucci *et al.* with permission.[14]

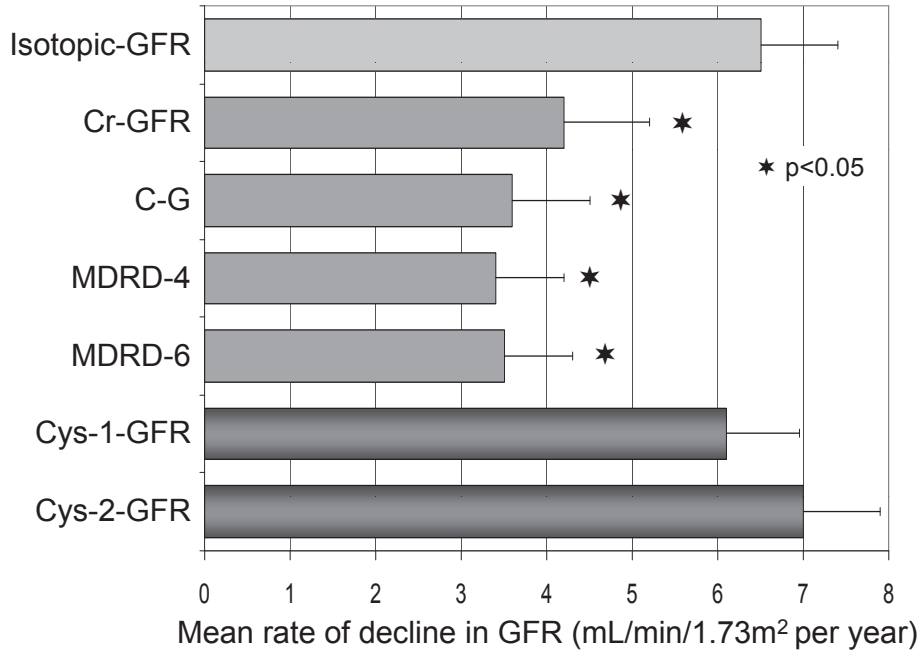

Figure 4. Comparison of the difference between the rates of decline in GFR (mL/min/1.73m^2 per year) as measured by isotopic-DTPA clearance and various indirect estimates of GFR in 19 patients with type 1 diabetes with a mean follow-up period of 10.1 years. Patients were selected for this analysis if they had a rate of GFR decline ≥3.3 mL/min/1.73m^2 per year. The initial reference-isotopic GFR was 105 and the final GFR was 52 mL/min/1.73m^2. Cr-GFR = 10^4/serum creatinine; C-G = Cockcroft-Gault formula; MDRD-4 = Modification of Diet in Renal Disease four-variable formula; MDRD-6 = Modification of Diet in Renal Disease six-variable formula; Cys-1-GFR = (86.7/serum cystatin C concentration) - 4.2, Cys-2-GFR = 100/cystatin C. Adapted from Premaratne *et al*. with permission.[6]

Summary

Recent work suggests that measurement of serum cystatin C concentration provides a simple and accurate method for detecting early renal impairment and for following trends in kidney function in subjects with diabetes. Although extra-renal factors may influence cystatin C concentration, the influence of these factors on cystatin C is likely to be much smaller than their corresponding effects on creatinine.

Whilst cystatin C is emerging as a very promising alternative to creatinine-based methods for estimating GFR, there remains a need to standardise its measurement, to calibrate it against an international standard and to further investigate the influence of non-renal factors on it. It is also necessary to develop reference intervals for cystatin C in large numbers of subjects with and without diabetes across a wide range of renal function. For these reasons and because of its cost, although cystatin C is well-established as a research tool, it is unlikely that it will make the transition to routine clinical practice in the near future. Furthermore, it has yet to be shown that estimating GFR from cystatin C compared with creatinine-based methods improves risk stratification for adverse vascular and renal outcomes in subjects with diabetes. In the meantime, creatinine-based methods will almost cetainly remain the most clinically applicable way of estimating GFR. However, the optimal method for transforming creatinine measurements so that they accurately estimate GFR across a wide range of kidney function is yet to be defined.

Competing Interests: None declared.

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