Cystatin C-based Equations in Renal Transplantation: Moving Toward a Better Glomerular Filtration Rate Prediction?

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Creatinine-based glomerular filtration rate (GFR) estimators perform poorly in renal transplant recipients. Cystatin C might be a better alternative to serum creatinine in assessing renal graft function. We compared several cystatin C-based equations with the modification diet renal disease (MDRD) equation in 120 adult renal transplant recipients for whom the GFR was measured by the gold standard inulin clearance. Mean inulin-measured GFR was 52.6 mL/min/1.73 m^2 (range, 13–119). The Hoek, Rule, Le Bricon, and Filler cystatin C-based formulas showed significantly better performances (accuracy 30% of 82%, 81%, 78%, and 71%), than the MDRD equation (58%, Mac Nemar test, $P<0.01$). Sensitivity to detect a GFR below 60 mL/min/1.73 m^2 was significantly higher for the Hoek and the Rule equations (0.95, 95% CI 0.91–1) than for the MDRD equation (0.76, 95% CI 0.67–0.85). These data confirm that cystatin C as a GFR marker offers significant advantages over creatinine in renal transplantation.

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Among the different glomerular filtration rate (GFR) predicting equations, the modification diet renal disease (MDRD) equations tend to perform better in renal transplant recipients. However, even these equations lack adequate reliability, especially in situations where a precise assessment of renal graft function is needed (*1–3*).

Serum cystatin C has long been touted as a possible alternative to serum creatinine, and is now deemed as a valid GFR marker (*4*, *5*). Numerous equations incorporating cystatin C value has been developed from various populations to give a direct GFR estimate (*5*).

Three recent studies have specifically examined the performance of several of these equations in kidney transplant recipients. Unfortunately, they led to divergent conclusions. While two of them (*6*, *7*) concluded that, as compared with their creatinine counterparts, cystatin C equations provide a more accurate prediction of GFR, the latest study failed to find any advantage of cystatin C (*8*).

In the present study, we sought to confirm or infirm the superiority of cystatin C-based GFR prediction in a cohort of stable renal transplant patients for whom a GFR measurement by the gold standard inulin clearance was available.

MATERIALS AND METHODS

One hundred twenty inulin clearances (urinary technique as previously described [*9*]) were selected from patients with a stable renal graft function and free from any medication interfering with creatinine tubular secretion.

GFR was estimated using the re-expressed four variables MDRD equation and five cystatin C-based equations.

The Re-Expressed Four Variables MDRD Equation

$$175 \times \text{serum creatinine (mg/dl)} - 1.154 \times \text{age(years)}^{\exp} \times (-0.203) \times 0.742 \text{ (if woman)} \times 1.210 \text{ (if African-American)}.$$

Serum creatinine concentrations were measured using the enzymatic assay CREA VITROS (Ortho-Clinical Diagnostics, Issy-les-Moulineaux, France) and calibrated to isotope dilution mass spectrometry (IDMS)-traceable values according to the manufacturer's instructions:

$$\text{IDMS-calibrated serum creatinine}_{\mu mol/l} = 0.9651 \times (\text{conventional serum creatinine}_{\mu mol/l}) - 11.348$$

Five Cystatin C-Based Equations

Filler equation (*10*):

$$\text{Log}\,(\text{GFR}_{ml/min/1.73\ m^2}) = 1.962 + [1.123 \times \log\,(1/\text{cystatin C})]$$

Le Bricon equation (*11*):

$$\text{GFR}_{ml/min/1.73\ m^2} = [(78) \times (1/\text{cystatin C})] + 4$$

Hoek equation (*12*):

$$\text{GFR}_{ml/min/1.73\ m^2} = -4.32 + (80.35 \times 1/\text{cystatin C})$$

Larsson equation (*13*):

$$\text{GFR}_{ml/min} = 77.24 \times (\text{cystatin C}^{-1.2623})$$

Rule equation (*14*):

$$\text{GFR}_{ml/min/1.73\ m^2} = 76.6 \times (\text{cystatin C})^{-1.16}$$

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Cystatin C (mg/L) was measured by an immunonephelometric method (N latex Cys C Nephelometer II, Dade-Berhing).

The predictive performance of the six GFR estimates was assessed according to the K/DOQI standards (*15*) with absolute and relative bias, precision (standard deviation of absolute bias) and accuracy 30% (proportion of GFR falling within 30% of the true GFR). Additionally, we analyzed the diagnostic performance of the equations, defined as their SENSITIVITY to detect a GFR of 60 mL/min/1.73 m^2.

Accuracy between the five cystatin C-based equations and the MDRD equation was compared using the Mc Nemar test (Bonferroni adjusted level of significance of 0.01).

RESULTS

The 120 patients (all whites except one Asian) included were mainly male (sex ratio=2.16), with a mean age and body mass index of 53 years (range 22–77) and 24 kg/m^2 (range 16–34), respectively at the time of the inulin clearance measurement. The median transplantation time at inclusion was 12 months (range 3–240). Causes of end-stage chronic renal disease were mostly non-diabetic glomerular diseases (45%) and all patients received a calcineurin inhibitor-based regimen (tacrolimus 77%), associated with mycophenolic acid (66%), azathioprin (9%), or everolimus (6%) and steroids (63%; mean dose 4.4 mg/day; range, 0–10). Mean serum creatinine and cystatin C values (±SD) were, respectively, 115 μmol/L (±47) and 1.7 mg/L (±0.6). The mean, standard deviation, median, and range of the different GFR estimates are shown in Table 1.

The performance criteria of the different GFR estimates are displayed in Table 2.

The five cystatin C equations showed a higher accuracy than the MDRD equation (Fig. 1). Except for the Larsson equation (Mac Nemar test of 2.6, $P=0.11$), all the cystatin C equations had an accuracy 30% significantly superior to the MDRD ($P<0.01$). Both the Hoek and the Rule equations had an accuracy 30% over 80%. These two equations were also the best to detect a GFR threshold of 60 mL/min/1.73 m^2 with a similar sensitivity of 0.95 (95% CI 0.91–1) as compared with 0.90 (0.84–0.97), 0.77 (0.68–0.86), 0.76 (0.67–0.85), and 0.71 (0.61–0.81) for the Larsson, Le Bricon, MDRD, and Filler equations, respectively.

DISCUSSION

A consensus is now emerging on the necessity to find valid alternatives to the existing creatinine-based GFR estimates in renal transplantation. Herein, we tested five GFR predicting equations that incorporate cystatin C as a GFR marker instead of serum creatinine and found that they all perform better in bias, precision, and accuracy when compared with the MDRD equation.

We deliberately chose the MDRD equation as the unique creatinine-based comparator since this equation has regularly showed the best level of performance in predicting

TABLE 1. Descriptive statistics for inulin clearance, MDRD equation, and 5 cystatin C-based GFR estimates

GFR (mL/min/1.73 m^2)	Mean±SD	Median	Range
Reference method			
Inulin clearance	52.6±19.4	52.0	13–119
Creatinine-based estimates			
MDRD	61.3±23.3	59.6	17–143
Cystatin C-based estimates			
Hoek	48.6±15.3	50.3	16–97
Larsson	46.6±18.5	48.6	12–112
Filler	57.6±18.6	59.4	19–119
Le Bricon	55.4±14.9	57.1	23–103
Rule	47.5±15.8	49.0	15–101

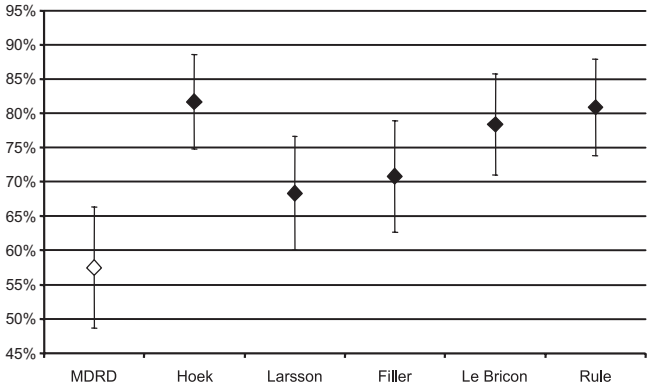

FIGURE 1. Accuracy 30% of GFR estimates (bars are 95% CI).

TABLE 2. Predictive performance of the MDRD equation and 5 cystatin C-based GFR estimates

GFR estimates	Bias		Precision	Accuracy 30%
	Absolute (mL/min/1,73 m^2)	Relative (%)	(mL/min/1.73 m^2)	(±95% CI)
Creatinine-based estimate				
MDRD	+8.7	+21.7	18.2	58 (49–67)
Cystatin C-based estimates				
Hoek	−4.0	−3.3	13.1	82 (75–89)
Larsson	−5.9	−8.8	15.4	68 (60–76)
Filler	+5.1	+14.6	13.9	71 (63–79)
Le Bricon	+2.8	+12.1	13.1	78 (71–86)
Rule	−5.0	−5.8	13.3	81 (74–88)

renal graft GFR when compared with other creatinine-based equations. Not surprisingly, in our population the five cystatin C equations were found to perform better than both the Cockcroft-Gault and Nankivell equations (data not shown).

The five cystatin C equations we selected have all been previously tested in renal transplant patients. Yet no definitive conclusion on their possible superiority over creatinine-based equations can be made. White et al. (*6*) and Poge et al. (*7*) agreed to conclude that these equations allow a more accurate GFR prediction than traditional creatinine-based equations. However, in these two studies, "true" GFR was determined using the plasma clearance of ^{99m}Tc-DTPA, which is not strictly equivalent to conventional urinary clearance (*16*). These data can thus be questioned and actually have been questioned. Using urinary clearance of inulin to measure GFR in 103 stable renal transplant patients, Zahran et al. (*8*) have conversely reported that the MDRD equation still gave a better prediction of renal graft function than any other cystatin C equations (accuracy 30% of 61% compared with at best 50% for the MDRD equation and the cystatin C equations, respectively).

Of note, this latest study is not immune from methodological critics either, and eventually does not help to close the debate. First, no appropriate calibration of serum creatinine measurement was performed. The importance of this issue has been extensively discussed for the MDRD equation (*17*, *18*) and has recently led to a revised equation that offers traceability to a reference method, the IDMS.(*19*) Because of the great susceptibility of the MDRD equation to the type of assays used to measure creatinine, calibration standardization either directly to the MDRD laboratory or to the IDMS reference is now absolutely required. In addition, Zahran et al. did not use one of the two admitted reference methods to measure serum cystatin C concentration, namely the particle-enhanced immunoturbidimetric assay and the immunonephelometric assay. Instead, they used an enzyme-linked immunosorbent assay method, which does not show optimal analytical performance. This certainly contributes to further limit the robustness of their conclusion.

In comparison, in the present study, we relied on (1) the gold standard inulin clearance to measure true GFR, (2) standardized serum creatinine values to predict GFR from the MDRD equation, and (3) the reference particle-enhanced immunonephelometric assay method to measure serum cystatin C. Using this stringent methodology, we confirm the better predictive performance of all cystatin C-based equations over the MDRD equation, with an added-value for the Hoek and the Rule equations that both gave an accuracy 30% over 80% and a sensitivity of 95% to detect a GFR over 60 mL/min/1.73 m^2.

Obviously, our study has also its own limitations. We think that its principal weakness resides on the rather limited number of included patients. Such sample size is only appropriate to provide a global picture of cystatin C-equations' performance but does not authorize relevant subgroup analyses.

Large-scale studies are thus necessary and should allow addressing several unresolved issues regarding the use of cystatin C in renal transplantation. Among them, two important questions must be thoroughly answered:

1. What are the transplantation-related factors susceptible to significantly interfere with serum cystatin C values? Rule reported a 19% higher GFR at the same cystatin C level among patients after renal transplantation in comparison to patients with native kidney disease, suggesting the intervention of factors specific with transplant patients (*14*). Indeed, immunosuppression by steroids and also by calcineurin inhibitors has been proposed to influence cystatin C values (*4*). The real impact of the different immunosuppressive regimens on cystatin C equations' accuracy is however unknown.
2. Which cystatin C equation is best qualified for renal transplant patients? Among the different cystatin C equations, there are some discrepancies between studies regarding which one is likely to perform the best in renal transplantation. While our data would favor the Hoek and Rule equations, the superiority of the Filler, Lebricon, and Larsson equations have been suggested by others (*6*, *7*). Consequently, it is currently premature, albeit tempting, to consider the equations that have been generated directly from a transplant cohort (i.e., the Lebricon and Rule equations) as the best candidates in renal transplantation.

In conclusion, the present study confirms the superiority of serum cystatin C over serum creatinine in predicting renal graft function. Our results, along with those of previous studies (*6*, *7*), call for a large-scale evaluation of cystatin C-based equations to further refine their clinical utility in renal transplantation.

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