



Published in final edited form as:

Hepatology. 2014 April ; 59(4): 1532–1542. doi:10.1002/hep.26556.

Performance of Chronic Kidney Disease Epidemiology Collaboration Creatinine-Cystatin C Equation for Estimating Kidney Function in Cirrhosis

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Abstract

Conventional creatinine-based glomerular filtration rate (GFR) equations are insufficiently accurate for estimating GFR in cirrhosis. The Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) recently proposed an equation to estimate GFR in subjects without cirrhosis using both serum creatinine and cystatin C levels. Performance of the new CKD-EPI creatinine-cystatin C equation (2012) was superior to previous creatinine- or cystatin C-based GFR equations. To evaluate the performance of the CKD-EPI creatinine-cystatin C equation in subjects with cirrhosis, we compared it to GFR measured by non-radiolabeled iothalamate plasma clearance (mGFR) in 72 subjects with cirrhosis. We compared the “bias”, “precision” and “accuracy” of the new CKD-EPI creatinine-cystatin C equation to that of 24-hour urinary creatinine clearance (CrCl), Cockcroft-Gault (CG) and previously reported creatinine- and/or cystatin C-based GFR-estimating equations. Accuracy of CKD-EPI creatinine-cystatin C equation as quantified by root mean squared error of difference scores [differences between mGFR and estimated GFR (eGFR) or between mGFR and CrCl, or between mGFR and CG equation for each subject] (RMSE=23.56) was significantly better than that of CrCl (37.69, P=0.001), CG (RMSE=36.12, P=0.002) and GFR-estimating equations based on cystatin C only. Its accuracy as quantified by percentage of eGFRs that differed by greater than 30% with respect to mGFR was significantly better compared to CrCl (P=0.024), CG (P=0.0001), 4-variable MDRD (P=0.027) and CKD-EPI creatinine 2009 (P=0.012) equations. However, for 23.61% of the subjects, GFR estimated by CKD-EPI creatinine-cystatin C equation differed from the mGFR by more than 30%.

CONCLUSIONS—The diagnostic performance of CKD-EPI creatinine-cystatin C equation (2012) in patients with cirrhosis was superior to conventional equations in clinical practice for estimating GFR. However, its diagnostic performance was substantially worse than reported in subjects without cirrhosis.

Keywords

End-stage liver disease; liver transplantation; cystatin C; glomerular filtration rate; gender disparity

INTRODUCTION

Cirrhosis was reported to be the twelfth leading cause of death in the U.S. along with chronic liver disease¹. It also constitutes one of the most common indications for liver transplantation². One of the most common and lethal complications of cirrhosis is hepatorenal syndrome characterized by an acute or subacute deterioration in renal filtration function³. Therefore, early diagnosis and treatment of kidney dysfunction in cirrhosis is of great importance.

Measuring glomerular filtration rate (GFR) by gold standard methods [e.g. renal clearance of inulin, ¹²⁵I-iothalamate or ^{99m}Tc-diethylene triamine pentaacetic acid (DTPA)] in clinical practice is laborious, time intensive and may involve radiation exposure. Several serum creatinine-based equations to estimate measured 24-hour urinary creatinine clearance (CrCl) and GFR have been developed including the Cockcroft-Gault (CG)⁴, 4- and 6-variable Modification of Diet in Renal Disease (MDRD) Study⁵ and Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) creatinine (2009)⁶ equations.

Several lines of evidence indicate that serum creatinine is not an accurate biomarker to detect kidney dysfunction in persons with cirrhosis. This inaccuracy is due to several factors: 1) synthesis of creatine, the precursor of creatinine, requires intact liver function⁷. Therefore, creatine production is reduced in cirrhosis⁷; 2) deconditioned subjects with cirrhosis with reduced skeletal muscle mass have disproportionately low serum creatinine levels relative to reductions in GFR⁷⁻⁹; 3) increased tubular secretion of creatinine in cirrhosis further lowers serum creatinine levels, resulting in underestimation of kidney dysfunction by CrCl⁷ and 4) spuriously low levels of serum creatinine are reported in subjects with hyperbilirubinemia and hemolysis¹⁰. Due to this low sensitivity of serum creatinine elevation, it is not surprising that both the CG⁴ and MDRD^{11, 12} equations have been shown to significantly overestimate GFR and thus mask renal impairment in subjects with cirrhosis¹³⁻¹⁵.

A more accurate equation than those that are creatinine-based GFR-estimating can provide a more sensitive means to detect early renal functional decline and lead to earlier and more successful treatment of this condition. In addition, it can correctly identify patients with cirrhosis and severe renal dysfunction that warrants consideration for simultaneous liver-kidney transplantation. It may also prevent iatrogenic injury (e.g. GFR-adjusted dosing can reduce drug-induced adverse events and prevent contrast-induced nephropathy). In recent years, an alternative biomarker, cystatin C has shown promise for estimating kidney function in cirrhosis. Cystatin C, a cysteine proteinase inhibitor is an ideal GFR marker as it has a constant secretion rate by all nucleated cells and due to its low molecular weight, passes freely through the glomeruli^{16, 17}. Almost the entire amount of cystatin C filtered by the glomeruli is reabsorbed and catabolized in proximal renal tubules^{16, 17}. Several equations based on serum cystatin C levels have been developed by Larsson *et al.*¹⁸ (LARSSON equation), Hoek *et al.*¹⁹ (HOEK equation) and Inker *et al.*²⁰ [CKD-EPI cystatin C equation (2012)] to estimate GFR. In recent equations by Stevens *et al.*²¹ (STEVENS equation) and Inker *et al.*²⁰ [(CKD-EPI creatinine-cystatin C equation (2012)], both serum creatinine and cystatin C, more accurately estimated kidney function compared to models that included serum creatinine or cystatin C alone in patients with a variety of disorders.

Few studies have used serum cystatin C to estimate kidney function in cirrhosis²²⁻²⁸. Plus, the equations developed by Stevens *et al.*²¹ and Inker *et al.*²⁰ have not been validated in subjects with cirrhosis.

To address these limitations, we tested the performance of the new CKD-EPI creatinine-cystatin C equation (2012)²⁰ to estimate GFR that directly measured by non-radiolabeled iothalamate clearance ('gold standard') in subjects with cirrhosis and compared its bias, precision and accuracy with that of CrCl, the CG⁴, creatinine-based [4-variable MDRD⁵, 6-variable MDRD5 and CKD-EPI creatinine (2009)⁶], cystatin C-based [LARSSON¹⁸, HOEK¹⁹ and CKD-EPI cystatin C (2012)²⁰] and creatinine-cystatin C-based (STEVENS²¹) GFR-estimating equations.

METHODS

Study Design and Participants

Subjects with cirrhosis were recruited from the University of Maryland Medical Center and University of Maryland Faculty Physicians, Inc. between September 2010 and March 2013. To be eligible, inclusion criteria included the following: presence of cirrhosis based on either liver biopsy or clinical, laboratory and radiological data; 18 years of age or older. Exclusion criteria included the following: inability to provide an informed consent; pregnancy or breastfeeding; iothalamate or iodine allergy; hepatocellular carcinoma that was not treated; hyperthyroidism (e.g. Graves' disease, multinodular goiter, thyroid autonomy or active treatment with radioactive iodine); inability to collect or void urine; being on dialysis or having an estimated GFR (eGFR) < 15 ml/min/1.73m²; treatment with corticosteroids within 1 week prior to enrollment; treatment with non-steroidal anti-inflammatory drugs (NSAIDs) except aspirin lower than 325 mg daily within 1 week prior to enrollment; treatment with angiotensin-converting enzyme (ACE) inhibitors within 1 week prior to enrollment; new onset of diuretics or dose change in diuretics within 1 week prior to enrollment; acute infection, exacerbation of encephalopathy, gastrointestinal bleeding and kidney injury within 1 week prior to enrollment; acute cardiovascular or cerebrovascular event within 3 weeks prior to enrollment; cognitive impairment.

The study was approved by the University of Maryland, Baltimore, Institutional Review Board (IRB). Written informed consent was obtained from all study participants.

Study Visits and Procedures

The study was conducted at the University of Maryland Medical Center, General Clinical Research Center and consisted of a screening (Visit 1) and procedure (Visit 2) visit. During the screening visit, a history and physical examination were performed. Every participant was educated on proper techniques for 24-hour urine collection and provided with a detailed patient education pamphlet. One day prior to the visit 2, each subject completed a 24-hour urine collection for CrCl and protein. One to three weeks later, the following procedures were performed at Visit 2 after an overnight fast (except diabetic patients who fasted only for 2 hours):

1) Blood and Urine Tests—Blood samples taken prior to the GFR measurement were analyzed for cystatin C, creatinine, basic metabolic panel [sodium, potassium, chloride, bicarbonate, blood urea nitrogen (BUN), creatinine and glucose], complete blood count, liver panel, coagulation tests and C-reactive protein (CRP). Urinalysis with microscopy was performed before the GFR measurement.

2) Measurement of GFR by Non-Radiolabeled Iothalamate Plasma Clearance—

GFR was measured using non-radiolabeled iothalamate plasma clearance. In order to minimize the risk of dehydration, subjects consumed 250 ml of water. At time 0, each subject had 1.5 ml of iothalamate (Conray-60, Mallinckrodt, St. Louis, MO) by intravenous infusion over 2 min and 3 ml of blood samples were drawn in heparinized tubes before and after iothalamate administration at 5, 15, 30, 45, 60, 120, 240 and 360 min

Laboratory Methods

Plasma Iothalamate Concentrations—Plasma for iothalamate concentration was harvested by centrifugation, aliquots were stored at -70°C until analysis and iothalamate concentrations were determined using reversed-phase high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection previously described by Dowling *et al.*²⁹.

Measurement of Serum Cystatin C and Creatinine Concentrations—Cystatin C was measured by a nephelometric technique using a Dimension Vista® System Flex® reagent cartridge (Siemens Healthcare Diagnostics, Newark, DE) was performed to measure serum cystatin C. Standardization of serum creatinine measurement technique was traceable to the isotope-dilution mass spectrometry (IDMS) materials³⁰.

Modeling Methods

A two-compartment model was used to model plasma iothalamate concentration data against time³¹. An iterative least-square method was performed to fit the model to the plasma iothalamate concentration data³¹ using WinNonlin® Version 5.1 (Certara L.P. (Pharsight), St. Louis, MO).

Statistical Analysis

Statistical analyses were performed using SAS software, Version 9.2 (Cary, NC)³² and Minitab statistical software (Minitab, Inc., State College, PA)³³. Categorical and quantitative variables were compared between groups using Chi-square or Fisher's exact tests and Wilcoxon two-sample test³⁴, independent t-test or one-way analysis of variance (ANOVA) test, respectively. Independent predictors of serum creatinine and cystatin C levels were assessed using multivariable linear regression analysis.

Performance of CrCl, CG Equation and GFR-Estimating Equations in Cirrhosis

—Body-surface area was calculated using formula developed by DuBois and DuBois³⁵. mGFR, CrCl, CG and LARSSON equations were corrected for body surface area.

The performance of CKD-EPI creatinine-cystatin C equation was compared to CrCl, the CG equation, creatinine-based (4- and 6-variable MDRD and CKD-EPI creatinine), cystatin C-based (LARSSON, HOEK, CKD-EPI cystatin C) and creatinine-cystatin C-based (STEVENS) GFR-estimating equations. The principal analyses were based on “difference scores”, i.e., the differences between mGFR and eGFR [(or the differences between mGFR and CrCl, or between mGFR and CrCl estimated by CG equation) for each subject. “Bias” of the CrCl, CG equation and GFR-estimating equations was determined by calculating the mean of difference scores, “precision” was determined by calculating the standard deviation of the difference scores³⁶. The overall “accuracy” of CrCl, CG and GFR-estimating equations for mGFR was determined by two different methods. First, we assessed the percentage of eGFRs that differed by greater than 20% (1- P₂₀) and 30% (1- P₃₀) with respect to mGFR as per Inker *et al.*²⁰. Second, we assessed the accuracy by the root mean squared error (RMSE), the square root of the mean of the squares of the difference scores³⁶.

Paired t-tests³⁶ were employed to determine whether CrCl, CG and GFR-estimating equations differed significantly from CKD-EPI creatinine-cystatin C equation with respect to bias, precision and accuracy assessed by RMSE. McNemar test³⁷ was used to determine whether CrCl, CG and GFR-estimating equations differed significantly from CKD-EPI creatinine-cystatin C equation with respect to accuracy assessed by (1- P₂₀) and (1- P₃₀).

ANOVA and independent t-tests were used to determine whether the performance of the CKD-EPI creatinine-cystatin C equation differed significantly among subgroups (subjects with pre-ascites, diuretic-sensitive ascites, and diuretic-refractory ascites; those with mGFR < 60 ml/min/1.73m² and mGFR ≥ 60 ml/min/1.73m²; and those with and without diabetes) with respect to bias, precision and accuracy assessed by RMSE. Chi-square and Fisher exact tests were used to determine whether CKD-EPI creatinine-cystatin C equation differed among subgroups with respect to accuracy assessed by (1- P₂₀) and (1- P₃₀).

RESULTS

Characteristics of 72 Subjects with Cirrhosis

Between September 2010 and March 2013, 85 subjects were enrolled of whom 72 completed the study. Table 1 and 2 show demographic, clinical and laboratory characteristics of 72 subjects. The majority of subjects (40%) had cirrhosis resulting from hepatitis C. Type of ascites was defined according to Arroyo *et al.*³⁸; 25 (35%) had no ascites (pre-ascites), 27 (37%) had diuretic-sensitive ascites and 20 (28%) had diuretic-refractory ascites. The MELD score was 6 to 9 in 22 (31%), 10 to 19 in 42 (58%) and ≥ 20 in 8 (11%) subjects. Only 4 subjects had urine protein > 0.5 g/24 hours.

Twenty-one subjects (29%) had mGFR < 60 ml/min/1.73m². There was a significant difference in mean mGFR among subjects with pre-ascites (106.9 ml/min/1.73m²), diuretic-sensitive ascites (75.8 ml/min/1.73m²) and diuretic-refractory ascites (64.8 ml/min/1.73m²) (P<0.0001). The mean mGFR was significantly lower in subjects with ascites compared to those with pre-ascites (71.1 vs. 106.9 ml/min/1.73m², P=0.0001).

The median serum creatinine level was significantly lower in women than men (0.7 vs. 0.9 mg/dl, P=0.015), but there was no significant difference in median cystatin C levels (1.0 vs. 1.1 mg/L, P=0.545) and mGFR (74.5 vs. 77.9 ml/min/1.73 m², P=0.973) between women and men. Controlling for age, female sex was an independent predictor of serum creatinine level (β =−0.205, P=0.007), but not of serum cystatin C level (β =−0.055, P=0.526). In a multiple regression analysis, only mGFR (β =−0.005, P<0.0001) was an independent predictor of serum cystatin C after controlling for age, presence of hepatitis C, ascites, diabetes, hypothyroidism. On the other hand, female sex (β =−0.175, P=0.007) in addition to mGFR (β =−0.004, P<0.0001) was an independent predictor of serum creatinine.

Median cystatin C levels did not differ between subjects with CRP levels ≤ 1.0 mg/dl and those with > 1.0 mg/dl (1.0 vs. 1.1 mg/L, P=0.286); between subjects with and without hypothyroidism (1.0 vs. 1.1 mg/L, P=0.484); between subjects with and without diabetes (1.1 vs. 1.1 mg/L, P=0.761); between subjects with and without hepatitis C cirrhosis (1.1 vs. 1.0 mg/L, P=0.370) and between African-Americans and non-African-Americans (1.1 vs. 1.0 mg/L, P=0.782).

Performance of CKD-EPI Creatinine-Cystatin C Equation (2012)

Table 3 shows the performance (bias, precision and accuracy) of CrCl, CG and GFR-estimating equations compared to that of the CKD-EPI creatinine-cystatin C equation in 72 study subjects.

Bias—Bias was estimated by the mean of difference scores (mGFR-eGFR, mGFR-CrCl or mGFR-CrCl estimated by CG equation). The CKD-EPI creatinine-cystatin C equation had lower bias than any other serum creatinine and/or cystatin C-based GFR-estimating equations (Table 3 and Figure 1). The direct measure of CrCl had the smallest bias but it was not significantly different from the CKD-EPI creatinine-cystatin C equation. Also, while the bias of the STEVENS equation was significantly different in value from the CKD-EPI creatinine-cystatin C equation, their magnitudes were similar, but in opposite direction. In general, creatinine-based equations overestimated mGFR, cystatin C-based equations underestimated mGFR.

Precision—Precision of CrCl, CG and GFR-estimating equations was estimated by the standard deviation of difference scores. Lower values indicate greater precision. As shown in Table 3, the combined creatinine-cystatin C-based equations including CKD-EPI creatinine-cystatin C and STEVENS equations had the highest precision. There was no significant difference in precision between the CKD-EPI creatinine-cystatin C and CG, 4- and 6-variable MDRD, CKD-EPI creatinine and STEVENS equations. Its superior precision is reflected in the relatively smaller interquartile range (smaller box-size) for CKD-EPI creatinine-cystatin C equation in Figure 1.

Accuracy—We evaluated accuracy by three statistical methods: 1- P_{30} (the percentage of CrCl, CrCl estimated by the CG equation and eGFRs that differed by greater than 30% with respect to mGFR), 1- P_{20} (the percentage of CrCl, CrCl estimated by CG equation and eGFRs that differed by greater than 20% with respect to mGFR) and RMSE. Lower values of these parameters indicated higher accuracy (Table 3). In 23.61% of subjects with cirrhosis, the CKD-EPI creatinine-cystatin C estimate differed from mGFR by more than 30% (1- P_{30}), and the other equations performed even more poorly. The accuracy of CKD-EPI creatinine-cystatin C equation measured by (1- P_{30}) was significantly better than CrCl and conventional equations including CG, 4-variable MDRD and CKD-EPI creatinine equations. In 44.44% of the subjects, the CKD-EPI creatinine-cystatin C estimate differed from mGFR by more than 20% (1- P_{20}). The accuracy of the CKD-EPI creatinine-cystatin C equation measured by (1- P_{20}) was only significantly better than the CG equation.

When we evaluated the accuracy by RMSE, CKD-EPI creatinine-cystatin C equation had the highest accuracy (lowest RMSE) that was significantly superior to the accuracy of CrCl, CG, LARSSON, HOEK and CKD-EPI cystatin C equations (Table 3).

We examined the performance of the CKD-EPI creatinine-cystatin C equation in subgroups defined by ascites (Table 4). For most measures, the equation performed best in the group with diuretic-sensitive ascites though none of the differences reached statistical significance.

We also examined the performance of the CKD-EPI creatinine-cystatin C equation in subgroups defined by mGFR level (Table 5). We found that precision and accuracy (measured by RMSE) were significantly superior in subjects with mGFR < 60 ml/min/1.73m² compared to those with mGFR ≥ 60 ml/min/1.73m².

There were no significant differences in bias (7.25 vs. -0.49, P=0.245), precision (26.45 vs. 21.64, P=0.411) and accuracy by (1- P_{30}) (22.73 vs. 24.00, P=1.000), (1- P_{20}) (45.45 vs. 44.00, P=0.910) and by RMSE (27.43 vs. 21.65, P=0.386) of the CKD-EPI creatinine-cystatin C equation between subjects with and without history of diabetes, respectively.

Performance Comparison of CKD-EPI Creatinine-Cystatin C Equation (2012) and 6-Variable MDRD Study Equation In Subjects with Cirrhosis Stratified by Type of Ascites

We compared the performance of CKD-EPI creatinine-cystatin C equation to 6-variable MDRD equation in subgroups defined by ascites (Table 6). Among subjects with diuretic-sensitive ascites, CKD-EPI creatinine-cystatin C equation had significantly lower bias (-1.11 vs. -8.60 , $P=0.019$), superior precision (15.36 vs. 19.24 , $P=0.037$) and accuracy (measured by RMSE) (15.41 vs. 21.08 , $P=0.030$) compared to 6-variable MDRD equation. There was no significant difference in overall accuracy between CKD-EPI creatinine-cystatin C and 6-variable MDRD equations among subjects with pre-ascites and diuretic-refractory ascites.

Performance Comparison of CKD-EPI Creatinine-Cystatin C Equation (2012) and 6-Variable MDRD Study Equation In Subjects with Cirrhosis Stratified by Level of mGFR

We also compared the performance of CKD-EPI creatinine-cystatin C equation to 6-variable MDRD equation in subgroups defined by mGFR level (Table 7). Among subjects with $mGFR < 60$ ml/min/1.73m², CKD-EPI creatinine-cystatin C equation had significantly lower bias compared to 6-variable MDRD equation (-5.11 vs. -10.04 , $P=0.028$). There was no significant difference in overall accuracy between CKD-EPI creatinine-cystatin C and 6-variable MDRD equations among subjects with $mGFR < 60$ ml/min/1.73m² and 60 ml/min/1.73m².

DISCUSSION

In this study of subjects with cirrhosis, we assessed the performance of the new CKD-EPI creatinine-cystatin C equation to estimate GFR measured by “gold standard” non-radiolabeled iothalamate clearance, and compared to CrCl, the CG equation, creatinine-based (4- and 6-variable MDRD and CKD-EPI creatinine), cystatin C-based (LARSSON, HOEK and CKD-EPI cystatin C) and creatinine-cystatin C-based (STEVENS) GFR-estimating equations. The accuracy of CKD-EPI creatinine-cystatin C equation was significantly better than that of CrCl, the CG, 4-variable MDRD, CKD-EPI creatinine, LARSSON, HOEK and CKD-EPI cystatin C equations. However, its accuracy in estimating GFR in subjects with cirrhosis [(1-P₃₀)=23.6, (1-P₂₀)=44.4 and RMSE=23.56] (Table 3) was markedly lower than that reported among external validation cohorts of participants without cirrhosis [(1-P₃₀)=8.5, (1-P₂₀)=22.8 and RMSE=0.162 to 0.200]²⁰ [(higher values for (1-P₃₀), (1-P₂₀) and RMSE indicate lower accuracy)]. In subgroup analyses, we did not find significant differences in the performance of CKD-EPI creatinine-cystatin C equation among subjects with pre-ascites, diuretic-sensitive and diuretic-refractory ascites. However, the CKD-EPI creatinine-cystatin C equation performed significantly better among subjects whose mGFR was < 60 ml/min/1.73m² compared to those whose mGFR ≥ 60 ml/min/1.73m².

It should be emphasized that no currently available creatinine- or cystatin C-based estimating equations were derived from subjects with cirrhosis. Nonetheless, the CG and MDRD equations are the most commonly used equations to estimate CrCl and GFR in clinical practice^{4, 11, 12}. The inferior accuracy of CrCl and creatinine-based GFR-estimating equations in subjects with cirrhosis is consistent with results of several prior studies¹³⁻¹⁵. With respect to the accuracy of cystatin C-based GFR-estimating equations in cirrhosis, Poge *et al.*²⁸ reported poor agreement between GFR measured by inulin clearance and cystatin-C based equations developed by Hoek *et al.*¹⁹ and Larsson *et al.*¹⁸. Stevens *et al.*²¹ showed that an equation based on both cystatin C and creatinine, age, sex and race provided a more accurate estimate of mGFR in subjects with chronic kidney disease compared to an equation based solely on cystatin C. To our knowledge, no study has evaluated the

performance of STEVENS equation in subjects with cirrhosis. In the present study, the CKD-EPI creatinine-cystatin C equation was similar in accuracy to the STEVENS equation that is also a combined creatinine-cystatin C based GFR-estimating equation.

There was no difference in accuracy of the CKD-EPI creatinine-cystatin C and 6-variable MDRD equations among subjects with cirrhosis, although the CKD-EPI creatinine-cystatin C equation performed better on all measures (Table 3). In addition, among subjects with diuretic-sensitive ascites, the accuracy of CKD-EPI creatinine-cystatin C equation was significantly superior to that of 6-variable MDRD equation (Table 6). Furthermore, among subjects with $mGFR < 60 \text{ ml/min/1.73m}^2$, the bias of CKD-EPI creatinine-cystatin C equation was significantly lower compared to that of 6-variable MDRD equation (Table 7). The 6-variable MDRD equation contains serum albumin in addition to serum creatinine, BUN, sex, age and race. Therefore, GFR estimates obtained using the 6-variable MDRD equation might have been influenced by albumin infusions among patients with cirrhosis and ascites. In addition to serum albumin, BUN can also be influenced by several factors⁵ and therefore the applicability of the 6-variable MDRD equation in cirrhosis is questionable.

Our results also indicate that cystatin C is a gender-neutral GFR marker in cirrhosis; while median serum creatinine level was significantly lower in women than men, there was no significant difference in median cystatin C levels nor $mGFR$ between women and men. Even after accounting for $mGFR$, age, hepatitis C, diabetes and hypothyroidism; women with cirrhosis had lower serum creatinine levels compared to men with cirrhosis. Controlling for the same factors, sex was not significantly associated with cystatin C levels. These results suggest that - among cirrhotic subjects- sex differences in creatinine concentration are not explained by renal filtration but by intrinsic differences in endogenous creatinine production. We previously showed that the use of serum creatinine in calculating MELD scores resulted in gender disparity on the liver transplant waiting list; women with end-stage liver disease had higher mortality compared to men⁹. Based on our results, it is possible that substitution of serum cystatin C for serum creatinine in the MELD equation would eliminate this gender disparity on the liver transplant waiting list.

We found moderately lower $mGFR$ in subjects with ascites compared to those with pre-ascites; the lowest mean $mGFR$ having been observed among subjects with diuretic-refractory ascites. The mean $mGFR$ was significantly lower in subjects with diuretic-sensitive and -refractory ascites compared to those with pre-ascites (71.1 vs. 106.9 ml/min/1.73m^2 , $P=0.0001$). Lower $mGFR$ among subjects with ascites might be due to chronically reduced renal blood flow even among the clinically stable patients in this study. The lack of significant proteinuria in 46 of 47 subjects with ascites is suggestive of lower renal blood flow as the cause of lower $mGFR$ rather than an intrinsic renal disease. Leslie *et al.*³⁹ showed that subjects with refractory ascites had reduced GFR. Other studies showed that renal resistive indices were significantly elevated in subjects with cirrhosis and ascites compared to those without ascites^{40, 41}. Increased renal resistive indices are suggestive of reduced renal blood flow in subjects with cirrhosis^{40, 41}. Our results are in line with these studies. This may predispose these patients to hepatorenal syndrome following diverse kidney insults such excessive diuresis, sepsis, frequent paracentesis, NSAIDs and ACE inhibitors.

This study has several strengths. Carefully structured inclusion and exclusion criteria enabled us to enroll a homogenous population of subjects with cirrhosis. Subjects with exposures that might cause acute changes in renal function (recent use of ACE inhibitors⁴² and NSAIDs⁴²) or influence cystatin C levels independently of eGFR (e.g. steroid use⁴³ and thyrotoxicosis⁴³) were excluded. GFR was measured by a well-accepted gold standard, non-radiolabeled iothalamate plasma clearance⁴⁴. IDMS traceable assay that was recommended

by the National Kidney Disease Education Program was used to standardize creatinine measurement³⁰. Plus, serum creatinine was measured with creatinase-enzymatic methodology that is less susceptible to interference by non-creatinine substances. Therefore, spuriously low serum or urine creatinine levels in subjects with cirrhosis with hyperbilirubinemia or hemolysis were avoided. Serum bilirubin levels greater than 9 mg/dl can potentially cause a negative bias exceeding 0.1 mg/dl or 10% of total with enzymatic creatinine methods⁴⁵; however only 3 subjects (4%) had bilirubin levels in excess of 9 mg/dl. Furthermore, we measured serum cystatin C by advanced nephelometric methods in a Clinical Laboratory Improvement Amendments (CLIA)-certified central laboratory. This method has negligible interference with bilirubin levels up to 60 mg/dl⁴⁶. Compared to using serum creatinine, this feature can potentially make cystatin C a more accurate GFR marker in subjects with cirrhosis with elevated bilirubin levels.

Study has some limitations. All participants were from a single center, and the sample size was smaller than some other validation studies of estimating equations. Nonetheless, our sample size (72 subjects) is as large as prior studies of renal function in subjects with cirrhosis, a population with unique challenges to recruitment and retention in clinical trials. The study population included Caucasian and African American subjects. Therefore, we were unable to assess the accuracy of the CKD-EPI creatinine-cystatin C equation (2012) in other races or ethnic groups. Importantly, 89% of our study population had low MELD score (MELD < 20) and a fairly well preserved renal function (71% with mGFR \geq 60 ml/min/1.73m² and only 1 subject with mGFR < 30 ml/min/1.73m²). Therefore, these results need to be interpreted with caution in subjects with more severe hepatic and renal dysfunction.

In conclusion, the overall diagnostic performance of the CKD-EPI creatinine-cystatin C equation in cirrhosis was superior to that of conventional, commonly-used methods including CrCl, CG and MDRD equations. However, the diagnostic performance of this equation was markedly lower than that reported in validation populations without cirrhosis. A more accurate cystatin C-based or combined creatinine-cystatin C-based GFR-estimating equation can be developed if the equation is specifically derived from subjects with cirrhosis. Until a more accurate GFR-estimating equation is developed to estimate kidney function in cirrhosis, we recommend the use of the CKD-EPI creatinine-cystatin C equation in place of conventional equations for early diagnosis and treatment of kidney dysfunction and prevention of drug-induced adverse events in subjects with cirrhosis.

Acknowledgments

The authors thank Charles Howell, M.D. (Professor of Medicine, Division of Gastroenterology and Hepatology, University of Maryland School of Medicine) and Jean-Pierre Raufman, M.D. (Professor of Medicine, Department of Medicine, Division of Gastroenterology and Hepatology, University of Maryland School of Medicine) for editing our manuscript and for their valuable input; Heather L. Rebeck, MT (ASCP), CLS (NCA) and Sharon Y. Huang, MT for analysis of blood samples, Myra T. Collins, B.A. (study coordinator) and the University of Maryland General Clinical Research Center Staff.

FUNDING

“The project described was supported by Grant Number 5 K23 DK089008-03 from the National Institutes of Health (NIH) National Institute of Diabetes and Digestive and Kidney Diseases (to Ayse L. Mindikoglu, M.D., M.P.H.) and its contents are solely the responsibility of the authors and do not necessarily represent the official views of the National Institute of Diabetes and Digestive and Kidney Diseases or the NIH”. This work was supported by the University of Maryland Clinical Translational Science Institute and the University of Maryland General Clinical Research Center.

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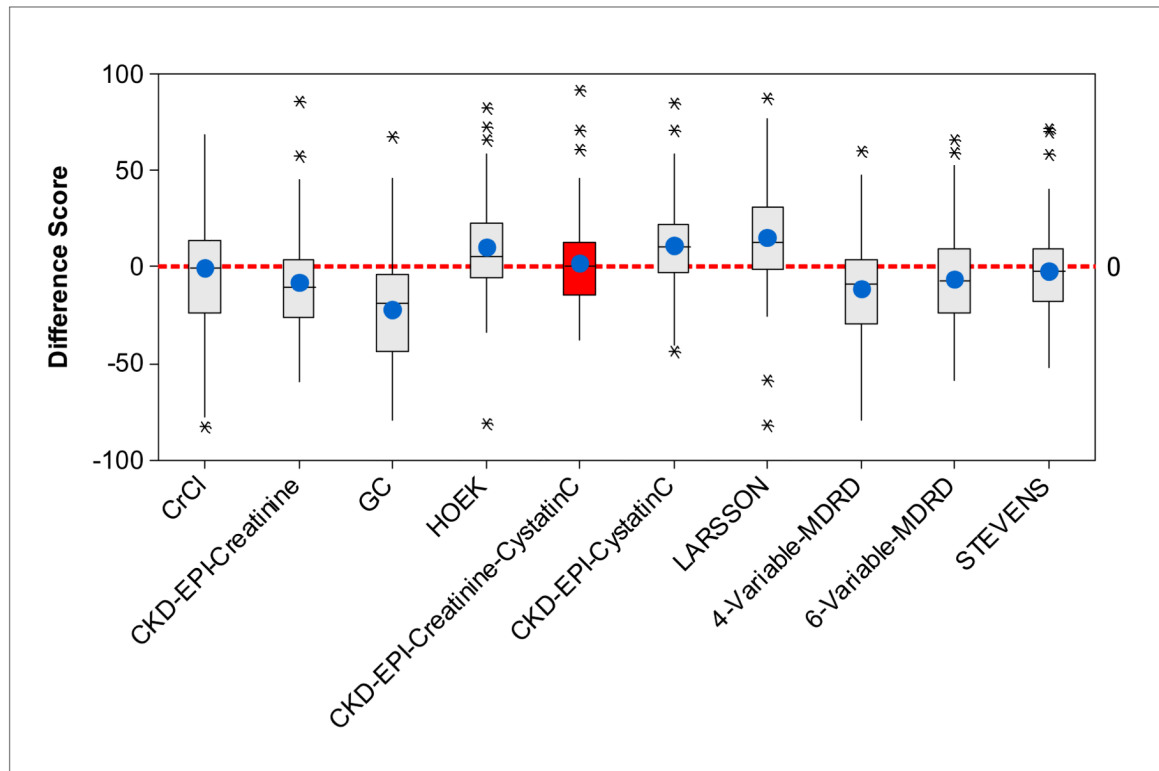


Figure 1.

Side-by-side boxplots of the difference scores [(DS)=mGFR-eGFR (or the differences between mGFR and CrCl, or between mGFR and CrCl estimated by CG equation) for each subject] for CrCl, CG and GFR-estimating equations; circles in each box show mean difference score (bias); black bars in each box show median difference score.

Table 1
Demographic, Clinical and Laboratory Characteristics of 72 Subjects with Cirrhosis

<i>Characteristics</i>	Number	%
Etiology of cirrhosis		
Hepatitis C	29	40
Hepatitis B	3	4
Alcohol	18	25
Nonalcoholic fatty liver disease	14	19
Primary biliary cirrhosis	2	3
Primary sclerosing cholangitis	1	1
Autoimmune hepatitis	3	4
Sarcoidosis	1	1
Sickle cell disease	1	1
Sex		
Female	37	51
Male	35	49
Race		
Caucasian	54	75
African-American	17	24
Asian	1	1
Ascites		
Pre-ascites	25	35
Diuretic-sensitive	27	37
Diuretic-refractory	20	28
MELD score		
6-9	22	31
10-19	42	58
20	8	11
C-reactive protein		
1.0 mg/dl	48	67
>1.0 mg/dl	24	33
Urine protein		
0.5 g/24 hours	68	94
>0.5 g/24 hours	4	6
Measured GFR		
90 ml/min/1.73m ²	27	38
60 and <90 ml/min/1.73m ²	24	33
30 and <60 ml/min/1.73m ²	20	28
15 and <30 ml/min/1.73m ²	1	1
Diabetes		
No	50	69
Yes	22	31

<i>Characteristics</i>		Number	%
Hypothyroidism			
	No	66	92
	Yes	6	8
		Median	IQR
<hr/>			
Age (yr)		54.0	12.0
Weight (kg)		80.6	27.2
Height (m)		1.7	0.1
Body-surface area (m ²)		1.9	0.3
MELD score		11.0	6.5
Serum creatinine (mg/dl)		0.8	0.4
Serum cystatin C (mg/liter)		1.1	0.5
Total bilirubin (mg/dl)		1.4	1.9
Prothrombin time (sec)		16.1	3.5
International normalized ratio		1.3	0.3
Serum albumin (g/dl)		3.1	0.8
BUN (mg/dl)		10.5	9.0
Serum sodium (mmol/liter)		137.0	5.0

IQR=Interquartile range

Table 2
Measured GFR, CrCl, Estimated CrCl (CG Equation) and Estimated GFR in 72 Subjects with Cirrhosis

Measured GFR*		83.6	35.0
CrCl*		84.1	42.1
Estimated CrCl*	CG Equation	106.3	39.0
Estimated GFR*	4- Variable MDRD Study equation	95.5	34.6
	6-Variable MDRD Study equation	89.9	33.0
	CKD-EPI creatinine equation (2009)	91.9	25.0
	LARSSON equation	68.2	29.9
	HOEK equation	73.2	26.4
	CKD-EPI cystatin C equation (2012)	72.4	27.7
	STEVENS equation	86.5	31.0
	CKD-EPI creatinine-cystatin C equation (2012)	81.7	26.5

* Expressed as ml/min/1.73m² of body surface area

Table 3
Bias, Precision and Accuracy of CrCl, CG and GFR-Estimating Equations in 72 Subjects with Cirrhosis

	CrCl and Creatinine-Based Equations				Cystatin C-Based Equations			Combined Creatinine-Cystatin C-Based Equations		
	CrCl	CG	4-Variable MDRD	6-Variable MDRD	CKD-EPI Creatinine (2009)	LARSSON	HOEK	CKD-EPI Cystatin C (2012)	STEVEN'S	CKD-EPI Creatinine-Cystatin C (2012)
Bias	-1.06	-22.78	-11.94	-6.39	-8.33	15.30	10.31	11.17	-2.90	1.87
P value [†]	0.447	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	
Precision *	37.67	28.03	25.77	24.07	24.68	30.44	28.06	26.56	23.44	23.49
P Value [‡]	0.001	0.119	0.320	0.753	0.357	0.016	0.045	0.004	0.961	
Accuracy (1-P₃₀) *	41.67	54.17	38.89	34.72	40.28	37.50	27.78	30.56	27.78	23.61
P Value [‡]	0.024	0.0001	0.027	0.077	0.012	0.099	0.629	0.359	0.375	
Accuracy (1-P₂₀) *	55.56	69.44	51.39	47.22	55.56	58.33	43.06	47.22	48.61	44.44
P Value [‡]	0.268	0.008	0.405	0.824	0.152	0.121	1.000	0.824	0.581	
Accuracy (RMSE) *	37.69	36.12	28.40	24.90	26.05	34.07	29.89	28.81	23.62	23.56
P Value [‡]	0.001	0.002	0.139	0.563	0.164	0.0004	0.005	0.0003	0.970	

mGFR=GFR measured by non-radiolabeled iothalamate plasma clearance (gold standard)

eGFR=Estimated GFR

Difference score (DS)=mGFR-eGFR (or the differences between mGFR and CrCl, or between mGFR and CrCl estimated by CG equation) for each subject

Bias=Mean DS

Precision=Standard deviation of DS

Accuracy=Percentage of eGFRs that differed by greater than 30% (1-P₃₀) or 20% (1-P₂₀) of mGFRs or root mean square error (RMSE)

* Lower values for precision and accuracy [(1-P₃₀), (1-P₂₀) and RMSE] indicate higher precision and accuracy for CrCl, CG and GFR-estimating equations

[†] P values compare the performance of CKD-EPI creatinine-cystatin C (2012) to CrCl, CG and GFR-estimating equations. Note, the P-val ues for bias reflect difference in the location of bias, not the magnitude. Thus, for example, there is a significant difference in the bias between the STEVEN'S and CKD-EPI creatinine-cystatin C equations that reflects differences in their location (negative vs. positive), but not their magnitude.

Table 4
Bias, Precision and Accuracy of CKD-EPI Creatinine-Cystatin C Equation (2012) in Subjects with Cirrhosis Stratified by Type of Ascites

	Pre-Ascites	Diuretic-Sensitive Ascites	Diuretic-Refractory Ascites	P Value [†]
Bias	10.67	-1.11	-5.09	0.058
Precision*	28.28	15.36	22.59	0.154
Accuracy (1-P₃₀)*	24.00	11.11	40.00	0.075
Accuracy (1-P₂₀)*	36.00	44.44	55.00	0.444
Accuracy (RMSE)*	30.23	15.41	23.16	0.141

mGFR=GFR measured by non-radiolabeled iothalamate plasma clearance (gold standard)

eGFR=Estimated GFR

Difference score (DS)=mGFR-eGFR (or the differences between mGFR and CrCl, or between mGFR and CrCl estimated by CG equation) for each subject

Bias=Mean DS

Precision=Standard deviation of DS

Accuracy=Percentage of eGFRs that differed by greater than 30% (1-P₃₀) or 20% (1-P₂₀) of mGFRs or root mean square error (RMSE)

* Lower values for precision and accuracy [(1-P₃₀), (1-P₂₀) and RMSE] indicate higher precision and accuracy for CrCl, CG and GFR-estimating equations

[†] P values compare the performance of CKD-EPI creatinine-cystatin C (2012) among subjects with pre-ascites, diuretic-sensitive and -refractory ascites. Note the low P-value for bias does not reflect a difference in magnitude of bias, but rather reflects (in this case) a difference in the direction of bias.

Table 5
Bias, Precision and Accuracy of CKD-EPI Creatinine-Cystatin C Equation (2012) in
Subjects with Cirrhosis Stratified by Level of mGFR

	mGFR \geq 60 ml/min/1.73m ²	mGFR < 60 ml/min/1.73m ²	P Value [†]
Bias	4.75	-5.11	0.035
Precision*	26.30	11.96	0.014
Accuracy (1-P₃₀)*	21.57	28.57	0.525
Accuracy (1-P₂₀)*	49.02	33.33	0.223
Accuracy (RMSE)*	26.72	13.01	0.011

mGFR=GFR measured by non-radiolabeled iothalamate plasma clearance (gold standard)

eGFR=Estimated GFR

Difference score (DS)=mGFR-eGFR (or the differences between mGFR and CrCl, or between mGFR and CrCl estimated by CG equation) for each subject

Bias=Mean DS

Precision=Standard deviation of DS

Accuracy=Percentage of eGFRs that differed by greater than 30% (1-P₃₀) or 20% (1-P₂₀) of mGFRs or root mean square error (RMSE)

* Lower values for precision and accuracy [(1-P₃₀), (1-P₂₀) and RMSE] indicate higher precision and accuracy for CrCl, CG and GFR-estimating equations

[†] P values compare the performance of CKD-EPI creatinine-cystatin C (2012) among subjects with mGFR \geq 60 ml/min/1.73 m² and mGFR < 60 ml/min/1.73m². Note the low P-value for bias does not reflect a difference in magnitude of bias, but rather reflects (in this case) a difference in the direction of bias.

Table 6
Bias, Precision and Accuracy of 6-Variable MDRD and CKD-EPI Creatinine-Cystatin C Equations in 72 Subjects with Cirrhosis Stratified by Type of Ascites

		6-Variable MDRD	CKD-EPI Creatinine-Cystatin C (2012)
Pre-Ascites	Bias	-5.14	10.67
	P value [†]	<0.0001	
	Precision*	30.06	28.28
	P Value [†]	0.578	
	Accuracy (1-P ₃₀) [*]	36.00	24.00
	P Value [†]	0.453	
	Accuracy (1-P ₂₀) [*]	48.00	36.00
	P Value [†]	0.453	
	Accuracy (RMSE) [*]	30.49	30.23
	P Value [†]	0.958	
Diuretic-Sensitive Ascites	Bias	-8.60	-1.11
	P value [†]	0.019	
	Precision*	19.24	15.36
	P Value [†]	0.037	
	Accuracy (1-P ₃₀) [*]	29.63	11.11
	P Value [†]	0.125	
	Accuracy (1-P ₂₀) [*]	48.15	44.44
	P Value [*]	1.000	
	Accuracy (RMSE) [*]	21.08	15.41
	P Value [†]	0.030	
Diuretic-Refractory Ascites	Bias	-4.97	-5.09
	P value [†]	0.968	
	Precision*	21.11	22.59
	P Value [†]	0.348	
	Accuracy (I-P ₃₀) [*]	40.00	40.00
	P Value [†]	1.000	
	Accuracy (I-P ₂₀) [*]	45.00	55.00
	P Value [†]	0.688	
	Accuracy (RMSE) [*]	21.69	23.16

		6-Variable MDRD	CKD-EPI Creatinine-Cystatin C (2012)
	P Value [†]	0.449	

mGFR=GFR measured by non-radiolabeled iothalamate plasma clearance (gold standard)

eGFR=Estimated GFR

Difference score (DS)=mGFR-eGFR for each subject

Bias=Mean DS

Precision=Standard deviation of DS

Accuracy=Percentage of eGFRs that differed by greater than 30% (1-P₃₀) or 20% (1-P₂₀) of mGFRs or root mean square error (RMSE)

* Lower values for precision and accuracy [(1-P₃₀), (1-P₂₀) and RMSE] indicate higher precision and accuracy for 6-Variable MDRD and CKD-EPI creatinine-cystatin C equations

[†] P values compare the performance of CKD-EPI creatinine-cystatin C (2012) to 6-variable MDRD equation. Note, the P-values for bias reflect difference in the location of bias, not the magnitude.

Table 7
Bias, Precision and Accuracy of 6-Variable MDRD and CKD-EPI Creatinine-Cystatin C Equations in 72 Subjects with Cirrhosis Stratified by Level of mGFR

		6-Variable MDRD	CKD-EPI Creatinine-Cystatin C (2012)
mGFR 60 ml/min/1.73m ²	Bias	-4.89	4.75
	P value [†]	0.0003	
	Precision*	27.08	26.30
	P Value [†]	0.721	
	Accuracy (I-P ₃₀) [*]	33.33	21.57
	P Value [†]	0.109	
	Accuracy (I-P ₂₀) [*]	47.06	49.02
	P Value [†]	1.000	
	Accuracy (RMSE) [*]	27.52	26.72
	P Value [†]	0.781	
mGFR < 60 ml/min/1.73m ²	Bias	-10.04	-5.11
	P value [†]	0.028	
	Precision*	13.65	11.96
	P Value [†]	0.473	
	Accuracy (I-P ₃₀) [*]	38.10	28.57
	P Value [†]	0.688	
	Accuracy (I-P ₂₀) [*]	47.62	33.33
	P Value [†]	0.453	
	Accuracy (RMSE) [*]	16.95	13.01
	P Value [†]	0.213	

mGFR=GFR measured by non-radiolabeled iothalamate plasma clearance (gold standard)

eGFR=Estimated GFR

Difference score (DS)=mGFR-eGFR for each subject

Bias=Mean DS

Precision=Standard deviation of DS

Accuracy=Percentage of eGFRs that differed by greater than 30% (1-P₃₀) or 20% (1-P₂₀) of mGFRs or root mean square error (RMSE)

* Lower values for precision and accuracy [(1-P₃₀), (1-P₂₀) and RMSE] indicate higher precision and accuracy for 6-variable MDRD and CKD-EPI creatinine-cystatin C equations

[†] P values compare the performance of CKD-EPI creatinine-cystatin C (2012) to 6-variable MDRD equation. Note, the P-values for bias reflect difference in the location of bias, not the magnitude.