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Estimation of Glomerular Filtration Rate in Patients with Cirrhosis by Using New and Conventional Filtration Markers and Dimethylarginines

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MEETING MATERIAL

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CONTRIBUTIONS

Ayşe L. Mindikoglu, M.D., M.P.H. The author was involved with study concept and design; acquisition of data; analysis and interpretation of data; drafting of the manuscript; critical revision of the manuscript for important intellectual content; statistical analysis; obtained funding; technical, or material support; study supervision.

Thomas C. Dowling, Pharm.D., Ph.D. The author was involved with analysis and interpretation of data, critical revision of the manuscript for important intellectual content.

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CONFLICT OF INTEREST

None to declare.

Abstract

Background and Aims—Equations used to estimate glomerular filtration rate (GFR) are not accurate in patients with cirrhosis. We aimed to develop a new equation to estimate the GFR in subjects with cirrhosis and compare its performance with chronic kidney disease epidemiology collaboration (CKD-EPI) cystatin C and creatinine-cystatin C equations, which were derived in populations without cirrhosis.

Methods—From 2010 through 2014, we measured GFR in 103 subjects with cirrhosis based on non-radiolabeled iothalamate plasma clearance. We measured blood levels of creatinine, cystatin C, beta-trace protein, beta-2 microglobulin, L-arginine, and symmetrical and asymmetrical dimethylarginines simultaneously with GFR. Multivariate linear regression analysis was performed to develop models to estimate GFR. Overall accuracy, defined by the root mean square error (RMSE) of our newly developed model to estimate GFR, was compared with that of the CKD-EPI equations. To obtain an unbiased estimate of our new equation to estimate GFR, we used a leave-one-out cross-validation strategy.

Results—After we considered all the candidate variables and blood markers of GFR, the most accurate equation we identified to estimate GFR included serum levels of creatinine and cystatin C, as well as patients' age, sex and race. Overall, the accuracy of this equation (RMSE=22.92) was superior to that of the CKD-EPI cystatin C equation (RMSE=27.27, $P=0.004$). Among subjects with cirrhosis and diuretic-refractory ascites, the accuracy of the equation we developed to estimate GFR (RMSE=19.36) was greater than that of the CKD-EPI cystatin C (RMSE=27.30, $P=0.003$) and CKD-EPI creatinine-cystatin C equations (RMSE=23.37, $P=0.004$).

Conclusions—We developed an equation that estimates GFR in subjects with cirrhosis and diuretic-refractory ascites with greater accuracy than the CKD-EPI cystatin C equation or CKD-EPI creatinine-cystatin C.

Keywords

End-stage liver disease; liver transplantation; prognostic factor; diagnostic

INTRODUCTION

Kidney disease is common in cirrhosis; its prevalence is estimated at 20% in hospitalized subjects with cirrhosis¹. Reliable glomerular filtration rate (GFR) markers to detect early kidney disease in cirrhosis are currently unavailable. Due to several factors, creatinine is not an accurate serum marker for glomerular filtration rate (GFR) in cirrhosis. Synthesis of creatine, the precursor of creatinine requires normal hepatic function and is compromised in decompensated cirrhosis resulting in reduced serum creatinine levels^{2, 3}. In patients with cirrhosis, diminished muscle mass and increased tubular secretion of creatinine further reduces serum creatinine levels²⁻⁶. In addition, hyperbilirubinemia and hemolysis in cirrhosis may spuriously lower creatinine levels^{7,8}.

In recent years, several novel GFR markers alternative to creatinine have emerged including cystatin C^{9,10}, beta-trace protein^{10,11}, beta-2 microglobulin¹⁰ and symmetric dimethylarginine (SDMA)^{12,13}. Cystatin C, beta-trace protein and beta-2 microglobulin¹⁰

have common characteristics. Due to their low molecular weight, they readily undergo filtration by glomeruli¹⁰, and do not have appreciable tubular secretion as is the case of creatinine¹⁰. More importantly, their production and release into serum is unaffected by hepatic dysfunction and is less influenced by age, sex and hyperbilirubinemia^{7,8,14}. SDMA is the isomer of asymmetrical dimethylarginine (ADMA), an endogenous inhibitor of nitric oxide synthase (NOS)^{12,13}. SDMA is produced by all nucleated cells and cleared by both the liver and kidneys^{15,16}. Siroen *et al.*¹⁷ demonstrated a significant negative correlation between SDMA levels and creatinine clearance in patients with alcoholic cirrhosis undergoing transjugular intrahepatic porto-systemic shunt placement ($P<0.001$). Luch *et al.*¹⁸ revealed a significant correlation between plasma SDMA and creatinine concentrations in patients with hepatorenal syndrome ($P<0.0001$); L-arginine/ADMA and L-arginine/SDMA ratios were reduced in subjects with cirrhosis and hepatorenal syndrome compared to healthy controls¹⁸. Additionally, Vallance *et al.*¹⁹ reported higher total concentration of SDMA and ADMA (SDMA+ADMA) in patients with renal failure compared to healthy controls. Mookerjee *et al.*²⁰ showed that (SDMA+ADMA) was significantly elevated in patients with acute alcoholic hepatitis plus cirrhosis compared to those who had cirrhosis alone. The results of these two studies in subjects with cirrhosis suggested that dimethylarginines might be a useful GFR marker in cirrhosis^{17–20}.

Measured GFR is a gold standard method to assess kidney function. However; currently available methods to measure GFR in subjects with cirrhosis (e.g. inulin, non-radiolabeled plasma or urinary iothalamate clearances, ¹²⁵I-iothalamate, ^{99m}Tc-diethylene triamine pentaacetic acid) are labor- and time-intensive, inconvenient, prone to urine collection errors, expose patients to radiation and consequently have not gained acceptance in clinical practice. Alternative methods to determine GFR include measuring 24-hour creatinine clearance (CrCl), estimating CrCl by the Cockcroft-Gault²¹ equation or estimating GFR by creatinine, cystatin C and creatinine-cystatin C-based equations^{14,21–27}. However, creatinine-based GFR equations underestimate the extent of kidney dysfunction in cirrhosis^{28–31}. Recently, De Souza *et al.*²⁸ showed that the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) cystatin C equation (2012) yielded the best results in subjects with cirrhosis regardless of the severity of ascites. We showed that compared to conventional creatinine- or cystatin C-based equations in cirrhosis, the CKD-EPI creatinine-cystatin C equation (2012)²³ was the most accurate for estimating GFR in cirrhosis³⁰. Nonetheless, its accuracy was substantially lower in subjects with cirrhosis than subjects without cirrhosis³⁰.

The objective of this present study was to derive a novel GFR-estimating equation in subjects with cirrhosis and assess its accuracy compared to measured GFR (mGFR) as well as existing CKD-EPI cystatin C (2012)²³ and creatinine-cystatin C (2012)²³ equations that were derived in populations without cirrhosis.

METHODS

Study Participants

Between September 2010 and September 2014, adult participants were recruited from University of Maryland Medical Center, University of Maryland Medical System and

University of Maryland, Baltimore, Faculty Physicians, Inc for two studies. Studies were approved by the Institutional Review Board of the University of Maryland, Baltimore.

Inclusion and Exclusion Criteria—Subjects were included in the studies if they were 18 years or older and had cirrhosis diagnosed clinically (based on clinical, radiological and laboratory data) or by liver histopathology. Subjects were excluded if cognitively impaired, pregnant or breastfeeding, allergic to iohalamate or iodine (or para-aminohippuric acid that was an additional exclusion criterion for the second study), on dialysis, treated with steroids or non-steroidal anti-inflammatory drugs except acetylsalicylic acid dose less than 325 mg daily within 1 week before enrollment. Subjects who were unable to consent or provide urine, with eGFR < 15 ml/min/1.73m², uncontrolled hyperthyroidism, transjugular intrahepatic porto-systemic shunt placement, previous kidney or liver transplant, acute cardiovascular and cerebrovascular event within 3 weeks before enrollment, acute gastrointestinal bleeding, acute kidney injury, encephalopathy exacerbation, acute infection, dose change in diuretics, angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers within 1 week before enrollment were also excluded.

Study Procedures

The studies consisted of three visits:

Visit 1—Subjects were scheduled for a 1-hour visit at the General Clinical Research Center or clinic. After obtaining written informed consent, a history and physical examination was performed. Subjects were instructed on the procedure for 24-hour urine collection for CrCl that started 24 hours prior to Visit 2.

Visit 2—One to three weeks after Visit 1, subjects were scheduled for a 6-hour procedure visit that consisted of GFR measurement, blood collection for GFR markers and laboratory tests. In 103 subjects, after an overnight fast (except diabetic subjects who fasted for 2 hours), GFR was measured by non-radiolabeled iohalamate clearance. Three ml of Conray-30 or 1.5 ml of Conray-60 was injected intravenously over 2 minutes. Five ml of blood was collected in heparinized tubes before and at 5, 15, 30, 45, 60, 120, 240 and 360 minutes after iohalamate injection. Blood samples for biomarkers were collected before and after GFR measurement. Blood was centrifuged and plasma was stored at –70°C until analysis.

Additionally, during the second study of 10 subjects with cirrhosis, renal plasma flow, renal resistive indices and renal markers were measured simultaneously with GFR and results were previously reported³².

Visit 3—This visit was scheduled one day after Visit 2 for 13 subjects whose GFR was measured by both plasma and urinary iohalamate clearance. In these subjects, 24-hour urine collection for urinary iohalamate clearance started simultaneously with plasma iohalamate clearance and was completed during this visit.

Methods

Measurement of GFR—Plasma and urine iothalamate concentrations were determined by reversed-phase high-performance liquid chromatography and ultraviolet detection described by Dowling *et al.*³³ Pharmacokinetic parameter estimates for iothalamate were calculated according to a two-compartment model³⁴ using iterative least-square method. For the analysis, WinNonlin® version 5.1 (Certara L.P. (Pharsight), St. Louis, MO) was used. MGFR was adjusted for body surface area estimated by the DuBois formula³⁵.

Measurement of Blood GFR Markers—Siemens Dimension Vista® System Flex® reagent cartridge (Siemens Healthcare Diagnostics Inc, Newark, DE) was used to determine serum creatinine, cystatin C and beta-2 microglobulin concentrations^{7,36,37}. Plasma beta-trace protein concentrations were determined at the University of Minnesota Advanced Research and Diagnostic Laboratory using N Latex β TP assay/Siemens ProSpec® nephelometer (Siemens Healthcare Diagnostics Inc, Newark, DE)³⁸. Plasma SDMA, ADMA and L-arginine concentrations were determined using the standard ELISA method of Diagnostika®^{15,39}.

Statistical Analysis

Statistical analyses were performed using SAS, Version 9.2 (Cary, NC)⁴⁰ and R software⁴¹. Minitab (Minitab, Inc., State College, PA)⁴² statistical software was used to construct Figure 2. The Fisher Exact test was used to compare categorical variables in pre-ascites, diuretic-sensitive and -refractory ascites groups. One-way analysis of variance (ANOVA) was performed to compare quantitative variables in ascites groups. Strength of correlation between GFR markers was measured by Spearman's rank-order correlation test. Univariate and multivariate linear analyses were performed to derive the novel equation. A linear GFR model was fit by regressing log of mGFR vs. log of GFR markers and candidate variables.

Cross-Validation of the New GFR-Estimation Equation—A direct estimate of the performance of the newly derived GFR-estimating equation using our internal patient data would result in biased estimates because the equations were derived to be optimal in our patients. To obtain an unbiased estimate of the accuracy of our new equations, we used leave-one-out cross-validation⁴³. In brief, we estimated GFR for each observation using a model fit based on all the other observations.

Performance of the New GFR-Estimating Equation—Performance (bias, precision and overall accuracy) of the new GFR-estimating, the CKD-EPI cystatin C and creatinine-cystatin C (2012) equations was estimated as follows^{30, 44}: First, for a given estimating approach, the difference between the mGFR (GFR measured by iothalamate plasma clearance) and eGFR (GFR calculated using the CKD-EPI cystatin C (2012), creatinine-cystatin C (2012) and new GFR-estimating equations) was calculated for each patient (i.e., mGFR-eGFR). Bias was defined as the mean of these difference scores so that a positive bias indicates that the eGFR was on average lower than the mGFR. Precision was defined as the standard deviation of the difference scores so that higher values indicate worse agreement; accuracy was defined as square root of the average squared difference score, i.e., the root mean squared error (RMSE). Higher values of RMSE indicate larger average

squared differences and therefore worse accuracy. Paired t-tests were performed to assess differences in bias, precision and accuracy between eGFR by CKD-EPI cystatin C, creatinine-cystatin C and new GFR-estimating equation^{30,44}. In calculating the eGFR from the two existing equations including CKD-EPI cystatin C and creatinine-cystatin C (2012) equations, we used the coefficients of these equations in Inker *et al.*²³

RESULTS

Demographic, clinical and Laboratory Characteristics

A total of 134 subjects with cirrhosis were enrolled; 103 subjects completed the studies (ninety-three subjects completed the first study and 10 completed the second study). Table 1 shows the demographic, clinical and laboratory characteristics of the 103 subjects overall and stratified by type of ascites. In each category of ascites, the proportion of subjects were similar; 31%, 37%, 32% in pre-ascites, diuretic-sensitive ascites and diuretic-refractory ascites, respectively. A majority of subjects had cirrhosis secondary to hepatitis C, followed by alcoholic, non-alcoholic fatty liver disease and other etiologies. Fifty-six percent of subjects were men. A majority of subjects were Caucasians (73%); 25% and 2% were African-Americans and Asians, respectively. Sixty-six percent of subjects had a MELD score between 10 and 19. C-reactive protein was elevated in 39% of subjects and significantly different among three ascites groups. The spot urine protein/creatinine ratio was 0.2 in 20% which may be indicative of glomerular disease. Twenty-nine and 11% of subjects had diabetes and hypothyroidism, respectively. MELD score, prothrombin time, international normalized ratio, serum albumin, basal urea nitrogen and sodium levels were significantly different among pre-ascites, diuretic-sensitive and -refractory ascites groups. Mean values for mGFR (Table 1, Figure 1) and CrCl (Table 1) were lower in subjects with diuretic-sensitive and -refractory ascites compared to those without ascites (pre-ascites) and significantly different in these three groups.

GFR Markers

Table 2 shows GFR markers including serum creatinine, cystatin C, beta-trace protein, plasma beta-2 microglobulin, SDMA, ADMA, L-arginine, L-arginine/SDMA, L-arginine/ADMA, (SDMA+ADMA) and L-arginine/(SDMA+ADMA). All GFR markers were significantly different in pre-ascites, diuretic-sensitive and -refractory ascites groups. While creatinine, cystatin C, beta-trace protein, beta-2 microglobulin, SDMA, ADMA, (SDMA+ADMA) were elevated in patients with diuretic-refractory ascites compared to patients with pre-ascites and diuretic-sensitive ascites, the ratios including L-arginine/SDMA, L-arginine/ADMA and L-arginine/(SDMA+ADMA) were lower compared to those with diuretic-sensitive ascites (Table 2).

Comparison of GFR Measured by Iothalamate Plasma and Urinary Clearances

Thirteen subjects with ascites (4 with diuretic-sensitive and 9 with diuretic-refractory) had simultaneous plasma and urinary iothalamate clearance to determine whether there was delay in plasma iothalamate clearance interfering with GFR measurements. There were no significant differences between the mean GFRs measured by plasma (57.1 ml/min/1.73m²)

and urinary iothalamate (61.5 ml/min/1.73m²) clearances (P=0.407). The similar plasma and urinary clearances provide evidence against significant “third-spacing” of this marker.

New GFR-Estimating Equation Developed from Subjects with Cirrhosis

There was a strong correlation between creatinine, cystatin C, beta-trace protein and beta-2 microglobulin. Spearman correlations ranged from 0.68 to 0.84 indicating that to a large degree, concentrations of these GFR markers reflected similar underlying excretory processes. Beta-trace protein, beta-2 microglobulin, SDMA, ADMA, (SDMA+ADMA), L-arginine/SDMA, and L-arginine/(SDMA+ADMA) were individually predictive of GFR, but not significantly informative when controlling for creatinine and cystatin C (**Table 3**). Therefore, there was no strong evidence that beta-trace protein, beta-2 microglobulin, SDMA, ADMA, (SDMA+ADMA), L-arginine/SDMA, and L-arginine/(SDMA+ADMA) were helpful for predicting mGFR in cirrhosis. Nonetheless, creatinine and cystatin C had the greatest associations with mGFR and were independently predictive of mGFR and despite their high correlation- both contributed significant information for the prediction of mGFR (**Table 3**). Relationships between GFR markers appeared linear on the log-scale (log mGFR vs. log GFR biomarkers). Therefore, a linear GFR model was fit by regressing log(mGFR) vs. log(serum creatinine), log(serum cystatin C), age, sex (female) and race (African-American). This new GFR-estimating equation resulted in the following model:

$$eGFR \text{ (ml/min/1.73m}^2\text{)} = 105.49 * (\text{serum creatinine}^{-0.712}) * (\text{serum cystatin C}^{-0.285}) * (0.993^{\text{age}}) * (0.864^{\text{female}}) * (1.014^{\text{African-American}})$$

Female is 1 if yes, 0 if no. African-American is 1 if yes, 0 if no.

Performance Comparison of the New GFR-Estimating Equation Developed from Subjects with Cirrhosis to CKD-EPI cystatin C and CKD-EPI Creatinine-Cystatin C Equations

In all subjects with cirrhosis, bias (P=0.001), precision (P=0.020) and overall accuracy (P=0.004) of the new GFR-estimating equation were superior to those of CKD-EPI cystatin C equation (**Table 4**). These differences were strongest in subjects with diuretic-refractory ascites and relatively small in subjects with pre- and diuretic-sensitive ascites (Table 4, Figure 2). In subjects with GFR ≥ 60 ml/min/1.73m², the precision (P=0.014) and accuracy (P=0.004) of the new GFR-estimating equation were superior to those of CKD-EPI cystatin C equation, but not to those of the CKD-EPI creatinine-cystatin C equation (**Table 5**).

Among subjects with diuretic-refractory ascites, the precision (P=0.002) and accuracy (P=0.004) of the new GFR-estimating equation was superior to those of the CKD-EPI creatinine-cystatin C equation (Table 4, Figure 2).

DISCUSSION

We recently reported that in subjects with cirrhosis, the accuracy of the CKD-EPI combined creatinine-cystatin C equation (2012) was superior to other widely-used estimating methods, including measured CrCl, the Cockcroft-Gault equation, and all conventional creatinine- or cystatin C-based GFR-estimating equations³⁰. It must be emphasized that none of these

equations were derived specifically from subjects with cirrhosis, a population in which accurate estimation of renal function is especially challenging. In the current study, using 103 subjects with cirrhosis, we developed a new GFR-estimating equation and compared its predictive performance to that of CKD-EPI cystatin C and creatinine-cystatin C equations derived in subjects without cirrhosis. Our new GFR-estimating equation outperformed the CKD-EPI cystatin C and creatinine-cystatin C equation (2012) in subjects with diuretic-refractory ascites; it also outperformed the CKD-EPI cystatin C equation in the entire cohort (**Table 4**). However, among those with $\text{mGFR} < 60 \text{ ml/min/1.73m}^2$, the accuracy of the new GFR-estimating equation was not significantly different from that of the CKD-EPI cystatin C and creatinine-cystatin C equations (**Table 5**).

In our study subjects with cirrhosis and diuretic-refractory ascites, the accuracy of CKD-EPI cystatin C and creatinine-cystatin C equations was markedly worse than their accuracy in the external validation datasets of subjects without cirrhosis reported by Inker *et al*²³. In our subjects with cirrhosis and diuretic-refractory ascites, RMSE for CKD-EPI cystatin C equation was 27.30 and RMSE for CKD-EPI creatinine-cystatin C equation was 23.37 (Table 3). In external validation datasets of subjects without cirrhosis, RMSE for CKD-EPI cystatin C equation was 0.175 to 0.265 and RMSE for CKD-EPI creatinine-cystatin C equation was 0.162 to 0.200²³ (these lower RMSE values indicate higher accuracy). The observation that the accuracy of the CKD-EPI cystatin C and creatinine-cystatin C equations was lower in subjects with cirrhosis compared to subjects without cirrhosis suggests that when developing a GFR-estimating equation for subjects with cirrhosis, it is critical to develop and test that equation in subjects with cirrhosis. Although the improved estimation of GFR using the new GFR-estimating equation was not substantial, such precision in estimating GFR has important implications. This level of improved accuracy may not be important in subjects with cirrhosis whose “true” GFR is well above $60 \text{ ml/min/1.73m}^2$. However, if a true GFR is borderline or low; small differences in accuracy could help providers make the safest decisions in daily clinical practice. These decisions may include frequent multi-drug dosing and administration of anesthesia drugs and contrast agents in subjects with cirrhosis⁴⁵. Accurate estimation of GFR is not only important for administering renally-adjusted drug dose, but also for determining whether to discontinue or hold medications (e.g. diuretics, ACE inhibitors). Moreover, the decision to proceed with simultaneous liver-kidney vs. liver transplantation alone is based in part on eGFR⁴⁶. Inaccurate estimation of GFR may lead to either underestimation of true GFR resulting in unnecessary kidney transplantation or overestimation of true GFR resulting in increased mortality after liver transplantation due to severe renal dysfunction⁴⁷.

Our study has several strengths. In developing the GFR-equation, we assessed all potential GFR markers including creatinine, cystatin C, beta-trace protein, beta-2 microglobulin, SDMA, ADMA, (SDMA+ADMA), L-arginine/SDMA, L-arginine/ADMA, and L-arginine/(SDMA+ADMA). We obtained the best performance by including both creatinine and cystatin C in our newly derived estimating equation (**Table 3**). We observed that while creatinine, cystatin C, beta-trace protein, beta-2 microglobulin, SDMA, ADMA and (SDMA +ADMA) were elevated in subjects with diuretic-refractory ascites compared to subjects with pre-ascites and diuretic-sensitive ascites, the ratios including L-arginine/SDMA, L-

arginine/ADMA and L-arginine/(SDMA+ADMA) were lower in subjects with diuretic-refractory ascites indicating accumulation of dimethylarginines in advanced cirrhosis (**Table 2**). In advanced cirrhosis, both SDMA and ADMA are associated with reduced nitric oxide (NO) production^{18,19,48}. NO is synthesized from L-arginine by NOS¹⁹. ADMA, an endogenous inhibitor of NOS^{18,19}, is hydrolyzed by dimethylarginine dimethylaminohydrolase (DDAH)^{16–20, 49–51}. Because DDAH activity requires intact liver function⁴⁸, ADMA levels are elevated in cirrhosis^{16–20,49–51}, thereby inhibiting NOS, reducing NO production and compromising renal plasma flow and GFR^{18,48}. In this setting, plasma levels of SDMA, an ADMA isomer, are also increased due to impaired hepatic and renal elimination^{16,18}. SDMA competes with L-arginine for endothelial transport^{16,48} and further reduces NO production, thereby further reducing renal plasma flow and GFR. Although our results related to dimethylarginines were in accord with prior studies, dimethylarginines were unable to predict GFR in cirrhosis as well as creatinine, cystatin C, beta-trace protein or beta-2 microglobulin. Therefore, our GFR model used the same GFR markers as the CKD-EPI creatinine-cystatin C equation.

Our study was limited primarily to Caucasian and African-American subjects. Therefore, we could not assess the applicability of the new GFR-estimating equation to Asians and other races. Only 9% of our study population had a MELD score ≥ 20 . External validation of the equation is required in subjects with higher MELD scores.

In conclusion, utilizing both serum creatinine and cystatin C we developed a novel GFR-estimating equation in subjects with cirrhosis; this equation had superior performance characteristics compared to previously-published CKD-EPI cystatin C and CKD creatinine-cystatin C (2012) equations in subjects with the combination of cirrhosis and diuretic-refractory ascites. We anticipate that after external validation, this novel equation will provide clinicians with a more accurate estimation of renal function in patients with cirrhosis and diuretic-refractory ascites, thereby facilitating more precise allocation in simultaneous liver-kidney transplantation.

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Figure 1.

GFR was measured in 103 subjects with cirrhosis. Mean values for mGFR were significantly different among subjects with pre-ascites, diuretic-sensitive and -refractory ascites ($P<0.0001$).

Figure 2.

Side-by-side boxplots of the difference scores in subjects with cirrhosis and diuretic-refractory ascites. Difference score= $mGFR - eGFR$ by CKD-EPI creatinine-cystatin C, CKD-EPI cystatin C and new GFR-estimating equations; black circles and lines in each box show mean (bias) and median difference score, respectively.

Table 1

Demographic, Clinical and Laboratory Characteristics of 103 Subjects with Cirrhosis

<i>Characteristics</i>	All		Pre-Ascites		Diuretic-Sensitive Ascites		Diuretic-Resistant Ascites		P Value
	N=103	%	N=32	%	N=38	%	N=33	%	
Etiology of cirrhosis									0.156
Hepatitis C	42	41	12	38	13	34	17	52	
Hepatitis B	3	3	2	6	1	3	0	0	
Alcohol	30	29	6	19	16	42	8	24	
Nonalcoholic fatty liver disease	18	17	7	22	6	16	5	15	
Primary biliary cirrhosis	2	2	1	3	1	3	0	0	
Primary sclerosing cholangitis	2	2	0	0	1	3	1	3	
Autoimmune hepatitis	3	3	3	9	0	0	0	0	
Sarcoidosis	1	1	0	0	0	0	1	3	
Sickle cell disease	1	1	1	3	0	0	0	0	
Hemochromatosis	1	1	0	0	0	0	1	3	
Sex									0.014
Male	58	56	13	41	20	53	25	76	
Female	45	44	19	59	18	47	8	24	
Race									0.231
Caucasian	75	73	23	72	24	63	28	85	
African-American	26	25	8	25	13	34	5	15	
Asian	2	2	1	3	1	3	0	0	
MELD score									0.002
6–9	26	25	16	50	7	18	3	9	
10–19	68	66	15	47	26	68	27	82	
20	9	9	1	3	5	13	3	9	
C-reactive protein									
1.0 mg/dl	63	61	26	81	22	58	15	45	0.010
>1.0 mg/dl	40	39	6	19	16	42	18	55	

		All		Pre-Ascites		Diuretic-Sensitive Ascites		Diuretic-Resistant Ascites		P Value
		N=103	%	N=32	%	N=38	%	N=33	%	
<i>Characteristics</i>										0.077
Urine protein										
	0.5 g/24 hours	87	93	26	90	34	100	27	87	
	>0.5 g/24 hours	7	7	3	10	0	0	4	13	
	Missing	9								
Spot urine protein/creatinine ratio										0.854
	<0.2	78	80	23	79	30	83	25	78	
	0.2	19	20	6	21	6	17	7	22	
	Missing	6								
Measured GFR										0.002
	90 ml/min/1.73m ²	34	33	17	53	12	32	5	15	
	60 and <90 ml/min/1.73m ²	35	34	12	38	11	29	12	36	
	30 and <60 ml/min/1.73m ²	32	31	3	9	15	39	14	42	
	15 and <30 ml/min/1.73m ²	2	2	0	0	0	0	2	6	
Diabetes										0.632
	No	73	71	22	69	29	76	22	67	
	Yes	30	29	10	31	9	24	11	33	
Hypothyroidism										0.211
	No	92	89	26	81	36	95	30	91	
	Yes	11	11	6	19	2	5	3	9	
		All		Pre-Ascites		Diuretic-Sensitive Ascites		Diuretic-Resistant Ascites		P Value
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Measured GFR*		80.28	35.16	100.70	36.98	77.14	29.71	64.08	28.93	<0.0001
Creatinine Clearance*		78.11	40.92	104.57	43.92	77.96	36.53	52.75	22.53	<0.0001
Age (yr)		54.53	8.88	54.88	8.58	53.61	10.46	55.27	6.89	0.714
Weight (kg)		83.67	19.26	86.91	17.58	79.24	20.45	85.63	18.44	0.201
Height (m)		1.69	0.09	1.67	0.09	1.68	0.09	1.71	0.09	0.241
Body-surface area (m ²)		1.93	0.23	1.95	0.19	1.88	0.24	1.97	0.23	0.234

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	All		Pre-Ascites		Diuretic-Sensitive Ascites		Diuretic-Resistant Ascites		
	N=103	%	N=32	%	N=38	%	N=33	%	P Value
Characteristics									
MELD score	12.88	4.66	9.84	3.39	14.08	4.62	14.45	4.34	<0.0001
Total bilirubin (mg/dl)	2.47	3.85	2.33	6.01	2.77	2.61	2.27	1.82	0.840
Prothrombin time (sec)	17.20	3.29	15.65	1.34	18.18	4.10	17.57	3.03	0.004
International normalized ratio	1.37	0.35	1.20	0.13	1.47	0.43	1.41	0.33	0.003
Serum albumin (g/dl)	3.08	0.55	3.32	0.52	3.12	0.55	2.80	0.43	0.0004
BUN (mg/dl)	14.46	8.68	10.72	4.95	13.47	6.39	19.21	11.28	0.0002
Serum sodium [mmol/L]	136.95	3.72	138.16	3.05	137.00	2.97	135.73	4.59	0.031

SD=Standard deviation

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

Table 2

GFR Markers in 103 Subjects with Cirrhosis

	All		Pre-Ascites		Diuretic-Sensitive Ascites		Diuretic-Resistant Ascites		P Value
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Creatinine (mg/dl)	0.90	0.34	0.71	0.21	0.89	0.28	1.10	0.40	<0.0001
Cystatin C (mg/L)	1.17	0.45	0.99	0.27	1.13	0.38	1.41	0.55	0.0004
Beta-trace protein (mg/L)	0.97	0.50	0.71	0.21	0.91	0.36	1.30	0.64	<0.0001
Beta-2 microglobulin (mg/L)	4.04	2.04	3.09	1.28	3.81	1.71	5.22	2.40	<0.0001
SDMA (micromol/L)	0.79	0.35	0.59	0.16	0.81	0.38	0.95	0.37	0.0001
ADMA (micromol/L)	0.66	0.18	0.57	0.12	0.65	0.14	0.76	0.23	<0.0001
SDMA+ADMA (micromole/L)	1.45	0.46	1.16	0.20	1.46	0.43	1.71	0.51	<0.0001
L-arginine (micromole/L)	78.94	27.97	66.37	20.44	89.01	28.85	79.53	28.44	0.003
L-arginine/SDMA	116.00	57.08	121.11	49.05	133.12	67.53	91.33	40.16	0.007
L-arginine/ADMA	125.66	49.09	120.42	40.76	143.33	54.11	110.38	43.80	0.014
L-arginine/(SDMA+ADMA)	58.67	24.83	58.86	20.35	67.17	29.11	48.71	19.11	0.007

SD=Standard deviation

Table 3

Univariate and Multivariate Linear Regression Models to Predict log(mGFR) in 103 Subjects with Cirrhosis

GFR Marker	Univariate Model that Includes only the log of the GFR Marker		Multivariate Model that Includes log(creatinine) and log(cystatin C) in addition to the GFR Marker ¹	
	R-Square for GFR Marker	P Value for GFR Marker	Partial R-Square for GFR Marker ²	P Value for the GFR Marker
log(creatinine)	0.573	<0.0001	0.119	<0.0001
log(cystatin C)	0.508	<0.0001	0.054	0.0002
log(beta-trace protein)	0.472	<0.0001	0.008	0.154
log(beta-2 microglobulin)	0.516	<0.0001	0.008	0.137
log(SDMA)	0.340	<0.0001	0.000	0.782
log(ADMA)	0.080	0.004	0.000	0.974
log(SDMA+ADMA)	0.306	<0.0001	0.000	0.816
log(L-arginine)	0.000	0.985	0.002	0.471
log(L-arginine/SDMA)	0.176	<0.0001	0.002	0.464
log(L-arginine/ADMA)	0.035	0.057	0.002	0.486
log[L-arginine/(SDMA+ADMA)]	0.126	0.0002	0.002	0.437

¹ For the model that include log(creatinine), log(cystatin C), and no other additional GFR marker but the variables including age, female and African-American, R-Square=0.675, P value for creatinine=<0.0001, P value for cystatin C=0.009

² Partial R-squares are interpretable as the additional proportion of variance explained by adding the GFR marker to a model that already includes creatinine and cystatin C

Table 4

Performance Comparison of New GFR-Estimating Equation to CKD-EPI Cystatin C and CKD-EPI Creatinine-Cystatin C Equations in All 103 Subjects with Cirrhosis and Stratified by Type of Ascites

		CKD-EPI Cystatin C Equation (2012)		CKD-EPI Creatinine-Cystatin C Equation (2012)		New GFR-Estimating Equation Developed from Subjects with Cirrhosis	
		Bias (95% CI)	P value [†]	Precision (95% CI) [*]	P Value [†]	Accuracy (95% CI) [*]	P Value [†]
All Subjects	Bias (95% CI)	7.43	(2.27 – 12.58)	–1.14	(–5.72 – 3.44)	2.54	(–1.94 – 7.01)
	P value [†]	0.001		0.0001			
	Precision (95% CI) [*]	26.37	(23.20 – 30.56)	23.44	(20.61 – 27.16)	22.89	(20.14 – 26.53)
	P Value [†]	0.020		0.589			
	Accuracy (95% CI) [*]	27.27	(21.22 – 32.21)	23.35	(18.13 – 27.60)	22.92	(17.12 – 27.53)
Pre-Ascites	P Value [†]	0.004		0.691			
	Bias (95% CI)	18.20	(7.70 – 28.70)	7.49	(–2.43 – 17.42)	12.03	(1.73 – 22.32)
	P value [†]	0.019		0.012			
	Precision (95% CI) [*]	29.12	(23.34 – 38.71)	27.53	(22.07 – 36.60)	28.55	(22.89 – 37.96)
	P Value [†]	0.830		0.634			
Diuretic-Sensitive Ascites	Accuracy (95% CI) [*]	33.95	(17.52 – 44.70)	28.12	(13.25 – 37.49)	30.57	(15.96 – 40.18)
	P Value [†]	0.196		0.275			
	Bias (95% CI)	1.97	(–4.65 – 8.59)	–5.84	(–11.64 – –0.03)	–1.11	(–6.98 – 4.76)
	P value [†]	0.236		0.006			
	Precision (95% CI) [*]	20.14	(16.42 – 26.06)	17.66	(14.40 – 22.85)	17.86	(14.56 – 23.11)
Diuretic-Refractory Ascites	P Value [†]	0.406		0.904			
	Accuracy (95% CI) [*]	19.97	(14.84 – 24.03)	18.38	(12.66 – 22.71)	17.66	(11.42 – 22.21)
	P Value [†]	0.423		0.639			
	Bias (95% CI)	3.26	(–6.49 – 13.02)	–4.11	(–12.39 – 4.18)	–2.46	(–9.38 – 4.45)
	P value [†]	0.040		0.263			
Diuretic-Refractory Ascites	Precision (95% CI) [*]	27.52	(22.13 – 36.40)	23.36	(18.79 – 30.90)	19.51	(15.69 – 25.80)
	P Value [†]	0.003		0.002			

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	CKD-EPI Cystatin C Equation (2012)		CKD-EPI Creatinine-Cystatin C Equation (2012)		New GFR-Estimating Equation Developed from Subjects with Cirrhosis	
	Accuracy (95% CI) *	P Value †	27.30 0.003	23.37 0.004	19.36 (11.52 – 24.84)	

Difference score for each subject=mGFR-eGFR by CKD-EPI cystatin C, CKD-EPI creatinine-cystatin C and new GFR-estimating equations

Bias=Mean of the difference scores

Precision=Standard deviation of the difference scores

Accuracy=Root mean squared error (RMSE)

* Lower values of precision and accuracy indicate higher precision and higher accuracy, respectively

† P values compare the performance of CKD-EPI cystatin C and CKD-EPI creatinine-cystatin C equations to new GFR-estimating equation. P-values reported for bias reflect difference in the location of bias, not the magnitude of bias

CI=Confidence interval

Table 5

Performance Comparison of New GFR Model to CKD-EPI Cystatin C and CKD-EPI Creatinine-Cystatin C Equations in All 103 Subjects with Cirrhosis and Stratified by Level of mGFR

	Bias (95% CI)	CKD-EPI Cystatin C Equation (2012)		CKD-EPI Creatinine-Cystatin C Equation (2012)		New GFR-Estimating Equation Developed from Subjects with Cirrhosis	
		10.02	(2.77 – 17.27)	1.14	(-5.22 – 7.50)	6.94	(0.83 – 13.05)
mGFR ≥ 60 ml/min/1.73m ²	P value [†]	0.127		<0.0001			
	Precision (95% CI)*	30.18	(25.85 – 36.27)	26.47	(22.67 – 31.81)	25.43	(21.78 – 30.55)
	P Value [‡]	0.014		0.426			
	Accuracy (95% CI)*	31.60	(23.93 – 37.73)	26.31	(19.64 – 31.59)	26.18	(18.76 – 31.91)
	P Value [‡]	0.004		0.927			
mGFR < 60 ml/min/1.73m ²	Bias (95% CI)	2.15	(-3.14 – 7.44)	-5.78	(-10.96 – -0.59)	-6.39	(-10.88 – -1.90)
	P value [‡]	<0.0001		0.503			
	Precision (95% CI)*	15.16	(12.23 – 19.96)	14.85	(11.98 – 19.55)	12.86	(10.37 – 16.93)
	P Value [‡]	0.240		0.030			
	Accuracy (95% CI)*	15.09	(10.58 – 18.54)	15.73	(7.11 – 21.07)	14.19	(5.40 – 19.33)
	P Value [‡]	0.699		0.125			

Difference score for each subject=mGFR-eGFR by CKD-EPI cystatin C, CKD-EPI creatinine-cystatin C and new GFR-estimating equations

Bias=Mean of the difference scores

Precision=Standard deviation of the difference scores

Accuracy=Root mean squared error (RMSE)

* Lower values of precision and accuracy indicate higher precision and higher accuracy, respectively

[‡] P values compare the performance of CKD-EPI cystatin C and CKD-EPI creatinine-cystatin C equations to new GFR-estimating equation. P-values reported for bias reflect difference in the location of bias, not the magnitude of bias

CI=Confidence interval