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Cystatin C is a Gender-Neutral Glomerular Filtration Rate Biomarker in Patients with Cirrhosis

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Laurence S. Magder, Ph.D., M.P.H. analyzed data, critically reviewed and revised the manuscript for important intellectual content.

Robert H. Christenson, Ph.D. conducted cystatin C and all other GFR biomarker measurements and critically reviewed the manuscript for important intellectual content.

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Abstract

Background—Lower serum Cr levels in women as compared to men result in underestimation of renal dysfunction and lower Model for End-Stage Liver Disease-Sodium (MELD-Na) scores leading to reduced access to liver transplantation in women compared to men with comparable hepatic dysfunction.

Aim—The aim of this study was to determine the gender differences in serum Cr, cystatin C and other endogenous GFR biomarkers, measured and estimated GFR, Cr clearance and Cr production rates.

Methods—We measured glomerular filtration rate (GFR) by iothalamate plasma clearance in 103 patients with cirrhosis and assessed gender differences in GFR, Cr clearance and production rate, serum Cr, cystatin C and other endogenous GFR biomarkers including beta-trace protein, beta-2 microglobulin and dimethylarginines.

Results—Comparison of men and women showed significantly lower values for mean serum Cr (0.97 vs 0.82 mg/dL, P=0.023), and Cr production rate (13.37 vs. 11.02 mg/kg/day, P=0.022). In contrast to the serum Cr and Cr production rate, men and women exhibited no significant differences in the means of serum cystatin C and other GFR biomarkers, measured GFR, GFR estimated using Cr-Cystatin C GFR Equation for Cirrhosis, measured and estimated Cr clearances. After controlling for age, race, weight, height and GFR, female gender remained associated with lower serum Cr levels (P=0.003). Serum cystatin C levels were not associated with gender, age, race, weight, height, C-reactive protein and history of hypothyroidism.

Conclusions—Our results suggest that cystatin C and endogenous GFR biomarkers other than Cr, measured GFR, GFR estimated by Cr-Cystatin C GFR Equation for Cirrhosis, measured and estimated Cr clearance minimized between-gender biases in accounting for renal function in patients with cirrhosis. Therefore, serum cystatin C should be measured as a complementary test to serum Cr when renal function is assessed in patients with cirrhosis, particularly in women and those with sarcopenia.

Keywords

Cystatin C; cirrhosis; glomerular filtration rate; gender disparity; creatinine clearance; liver transplantation

INTRODUCTION

Serum creatinine (Cr) is recognized to be an inaccurate marker of renal function in patients with cirrhosis.[1–4] The Cr production rate in patients with cirrhosis is about 50% lower than that in individuals with intact hepatic function.[1] The liver is the principal organ in which guanidinoacetic acid is methylated to produce methylguanidinoacetic acid (creatine)[1, 5]. Subsequently, creatine and creatine phosphate are converted to Cr in skeletal muscle.[1, 5] Therefore, optimal Cr production rate depends on both normal hepatic function and skeletal muscle mass.[1, 5] As a consequence of hepatic dysfunction and skeletal muscle mass loss (sarcopenia), the rate of Cr production rate is reduced in patients with cirrhosis.[1–3]

Cr production is also impacted by gender differences. The Cr production rate was reported to be about 10% lower in healthy women compared to age- and weight-matched healthy men.[5] The Model for End-Stage Liver Disease-Sodium (MELD-Na) score is used in the U.S. to allocate donor livers to patients with cirrhosis who have the greatest risk of mortality without liver transplantation.[6–8] The MELD-Na score is calculated using four laboratory tests: total bilirubin, prothrombin international normalized ratio, serum sodium and serum Cr.[6–8] Gender disparities in the allocation of donor livers to men vs. women on the liver transplant waiting list was previously documented.[9–14] (*Note: throughout this manuscript, the term 'gender' refers to binomial biological denomination of male and female sex without consideration of cultural or behavioral factors*). We previously conducted a competing risk survival analysis of the Organ Procurement and Transplantation Network (OPTN) database including 42,322 patients on the liver transplant waiting list, and showed that women with cirrhosis had significantly higher risk of dying within 3 years of listing compared to men ($P<0.0001$).[10] This analysis showed that the liver transplantation rate was systematically lower for women compared to men for almost each level of MELD score on the liver transplant waiting list.[10] One of the reasons for this biological gender disparity was that lower serum Cr levels in women with cirrhosis contribute to lower MELD scores resulting in reduced access to donor livers and significantly higher liver transplant waiting list mortality compared to men with comparable hepatic dysfunction.[10, 12, 13] Lower transplantation rates in women could also be due to transplant candidate/donor liver size mismatch in addition to lower MELD scores.[15] However, our prior analysis of the OPTN database showed that the contribution of the liver size to gender disparity was relatively small when compared to MELD score.[15] Our results were in line with the study by Lai *et al.*[9] that showed that low height contributed in part to gender disparity on the liver transplant waiting list however adjustment for height could not eliminate lower transplantation rates in women compared to men.[9, 15]

In recent years, new endogenous glomerular filtration rate (GFR) biomarker alternatives to Cr have been reported. The most thoroughly evaluated of these biomarkers include cystatin C, beta-trace protein, beta-2 microglobulin and symmetric dimethylarginine (SDMA).[16–21] Cystatin C is a small molecular weight protein produced by nucleated cells at a constant rate.[18] Its renal handling suggests it can be utilized as an endogenous marker for GFR.[18] Like Cr, it is freely filtered through the glomeruli, but unlike Cr, cystatin C is reabsorbed and metabolized in renal tubules rather than being secreted by renal tubules.[18] Cystatin C is a renal function biomarker in patients with cirrhosis because its production appears to be less dependent on hepatic function, age, gender or muscle mass.[18] However, a prior population-based study suggested that cystatin C levels were at least modestly affected by gender, age, weight and height, smoking and levels of C-reactive protein, a biomarker of inflammation independent of Cr clearance[22]. Importantly, cystatin C was shown to accurately predict GFR or Cr clearance in patients with cirrhosis.[23–26]

Like cystatin C, the low molecular weight GFR biomarkers beta-trace protein and beta-2 microglobulin are less affected by age, sex and race.[19, 27] However, neither was superior to cystatin C for prediction of GFR.[24, 27–29] SDMA is another alternative GFR biomarker produced by all nucleated cells and cleared by both the liver and kidneys.[21, 30] SDMA is an isomer of asymmetrical dimethylarginine (ADMA).[21] Studies conducted in patients with

cirrhosis reported significant correlation of SDMA levels with renal dysfunction and severity of cirrhosis.[24, 31–33] Our previous work showed that cystatin C, beta-trace protein, beta-2 microglobulin, and SDMA were significant predictors of measured GFR in cirrhosis.[24] However, among all these GFR biomarkers, only serum cystatin C, when used in combination with serum Cr, significantly increased the performance of the GFR equation.[24]

Given the adverse consequences of underestimation of renal dysfunction in the management of women with cirrhosis (e.g. drug overdose, excessive diuresis, late referrals for dialysis) and discriminatory disadvantage of women on the liver transplant waiting list caused by reliance on serum Cr, we conducted a prospective study to determine the gender differences in serum Cr, cystatin C and other endogenous GFR biomarkers, measured and estimated GFR, Cr clearance and Cr production rates.

METHODS

Study Subjects

This study was approved by the University of Maryland, Baltimore Institutional Review Board. Study procedures were conducted at the General Clinical Research Center and outpatient clinics of the University of Maryland Medical Center, Baltimore, Maryland between 2010 and 2016. Statistical analysis of this study was conducted at Baylor College of Medicine, Houston, TX after approval from Baylor College of Medicine Biomedical Research and Assurance Information Network (BRAIN) was obtained. Inclusion and exclusion criteria for the study were previously described.[24] All subjects enrolled in the study provided written consent.

Study Procedures

GFR was measured using iothalamate plasma clearance after intravenous bolus administration as described previously.[24, 32, 34] Twenty-four hour Cr clearance was measured starting one day prior to the GFR procedure and completed the morning of the GFR procedure. Measured GFR and measured Cr clearance were adjusted for body surface area using the formula developed by DuBois *et al.*[35]

GFR biomarker concentrations including Cr, cystatin C, beta-trace protein and beta-2 microglobulin were measured simultaneously with GFR measurement prior to iothalamate injection. Serum Cr, cystatin C and plasma beta-2 microglobulin concentration measurements were performed on the Siemens Dimension Vista® System, using the appropriate analyte specific Flex® reagent cartridges (Siemens Healthcare Diagnostics, Inc., Newark, DE). The serum Cr measurement method was calibrated to be traceable to the isotope-dilution mass spectrometry (IDMS) reference measurement procedure.[36] Serum beta-trace protein concentration measurements were conducted on the Siemens ProSpec® nephelometer using N Latex beta-trace protein assay (Siemens Healthcare Diagnostics, Inc., Newark, DE) at the University of Minnesota Advanced Research and Diagnostic Laboratory. Plasma SDMA, ADMA, and L-arginine concentrations were measured using the standard enzyme-linked immunosorbent method of Diagnostika (Hamburg, Germany).

Statistical Analysis

Statistical analyses were performed using SAS Version 9.4 TS level 1M3 W32_7PRO platform (SAS, Cary, NC).[37] The t-test was used to compare differences in continuous variables and Fisher's exact test was used to compare differences in categorical variables between men and women. A P value ≤ 0.05 was considered significant. The measured Cr production rate (mg/kg/day) was calculated using measured Cr clearance and serum Cr. The estimated Cr production rate was calculated using the Mitch and Walser equation.[38]. Estimated Cr clearance was calculated using the estimated Cr production rate using the Mitch and Walser equation[38] and serum Cr. Estimated GFR was calculated using the Cr-Cystatin C GFR Equation for Cirrhosis.[24] The equations used to calculate measured and estimated Cr production rates, estimated Cr clearance and estimated GFR were as follows:

Measured Cr production rate (mg/kg/day)[5] = (Measured Cr clearance in ml/min * serum Cr in mg/dL * 1440 min)/(weight in kg * 100 ml);

Estimated Cr production rate in men (mg/kg/day) using Mitch and Walser equation[38] = 28 − (0.2 * age);

Estimated Cr production rate in women (mg/kg/day) using Mitch and Walser equation[38] = 23.8 − (0.17 * age);

Estimated Cr clearance (ml/min/1.73m^2)= (Estimated Cr production rate in mg/kg/day using Mitch and Walser equation[38])*(weight in kg*100 ml)*(1.73m^2)/(serum Cr in mg/dl) * (1440 min) * (body surface area in m^2).

Estimated GFR in ml/min/1.73m^2 using Cr-Cystatin C GFR Equation for Cirrhosis[24]=105.49 * ($Cr^{-0.712}$) * (cystatin $C^{-0.285}$) * (0.993^{age}) *(0.864^{female}) * ($1.014^{African\text{-}American}$)

To determine the factors associated with the concentration of each endogenous GFR marker independent of measured GFR, a separate multivariate linear regression model was developed and controlled for age, race, weight, height and measured GFR.

The performance characteristics including bias, precision and accuracy of the measured and estimated Cr clearances were evaluated with respect to their ability to estimate measured GFR.[24, 34, 39, 40] First, the difference score for each subject (difference score=measured GFR-measured Cr clearance or difference score=measured GFR-estimated Cr clearance) was calculated. The bias and precision were defined as the mean and standard deviation of the difference scores, respectively. Negative values of the bias indicate that the measured and estimated Cr clearances were on average higher than measured GFR (overestimation of measured GFR). Higher precision values indicate more variation in the difference between measured GFR and Cr clearances. The accuracy of the measured and estimated Cr clearances were calculated by three different methods including (1- P_{30}) which is the percentage of Cr clearances that differed by greater than 30% with respect to measured GFR, (1- P_{20}) which is the percentage of Cr clearances that differed by greater than 20% with respect to measured GFR and root-mean-squared error (RMSE) which is the square root of the mean of the squares of the difference scores[39, 40]. Paired t-tests[40] were performed to determine whether estimated Cr clearance differed significantly from measured Cr

clearance with respect to bias, precision and accuracy assessed by RMSE. McNemar test[61] was performed to determine whether estimated Cr clearance differed significantly from measured Cr clearance with respect to accuracy assessed by (1- P_{20}) and (1- P30).

RESULTS

A total of 134 patients with cirrhosis were enrolled in the study; among them 103 patients completed study procedures. Table 1 shows the clinical, demographic and laboratory characteristics of 103 patients with cirrhosis stratified by gender. Forty-four percent were women. As expected, women were significantly shorter than men (1.62 vs. 1.73 m, $P < 0.0001$) and had significantly smaller body surface area (1.83 vs. 2.00 m, $P = 0.0001$), but had similar age, race, and measured GFR.

Table 2 shows gender differences in the mean measured GFR, GFR estimated using Cr-Cystatin C GFR Equation for Cirrhosis, measured and estimated Cr clearance, measured and estimated Cr production rate, serum Cr, cystatin C and other endogenous GFR biomarkers alternative to serum Cr. While there were significant differences in the mean serum Cr levels (0.97 mg/dL vs. 0.82 mg/dL, P=0.023), measured (13.37 vs. 11.02 mg/kg/day, P=0.022) and estimated Cr production rates (17.12 vs. 14.50 mg/kg/day, P<0.0001) between men and women, there were no significant differences in the mean measured GFR, GFR estimated using Cr-Cystatin C GFR Equation for Cirrhosis, measured and estimated Cr clearance, cystatin C and other endogenous GFR biomarkers alternative to serum Cr.

To determine the association of each endogenous GFR marker with female gender, a separate multivariate linear regression model was developed and controlled for age, race, weight, height and measured GFR (Table 3). Even after controlling for age, race, weight, height and measured GFR, female gender remained associated with lower serum Cr (β=−0.195, P=0.003), while female gender did not correlate with cystatin C (β=−0.130, P=0.169), beta-trace protein (β=−0.080, P=0.463), beta-2 microglobulin (β=−0.616, P=0.161), and SDMA (β=−0.107, P=0.184) levels (Table 3). In addition to serum Cr, female gender was also significantly associated with lower ADMA levels (β=−0.092, P=0.044).

To explore further the factors associated with serum cystatin C levels, we performed a multivariate analysis. The multivariate model showed measured GFR was independently associated with serum cystatin C levels, as expected (β = − 0.008, $P < 0.0001$). However, it did not reveal significant associations between cystatin C levels and age, gender, race, weight, height, C-reactive protein levels and history of hypothyroidism, independent of measured GFR (Table 4).

As we did not find significant difference in the mean values of measured and estimated Cr clearance between male and female subjects, we considered estimated Cr clearance as one of the practical alternative methods to replace serum Cr in the MELD-Na equation to eliminate gender disparity on the liver transplant waiting list in addition to non-Cr GFR biomarkers. Table 5 shows the performance measures of measured and estimated Cr clearances in reference to their ability to estimate measured GFR. We observed a larger bias between estimated Cr clearance and measured GFR compared to bias between measured Cr clearance

and measured GFR (− 23.15 vs. 1.13). There was a significant difference in the direction of bias between measured and estimated Cr clearances (P<0.0001). While measured Cr clearance slightly underestimated measured GFR, estimated Cr clearance substantially overestimated measured GFR. Lower values for precision and accuracy indicated higher precision and accuracy. The precision of estimated Cr clearance was higher compared to the precision of measured Cr clearance (26.72 vs. 34.43); however, this was not significant. The accuracy was calculated by using three different methods. We did not observe significant differences in the percentage of measured and estimated Cr clearance values that differed by greater than 30% (1-P_{30}) with respect to measured GFR (42.86 vs. 56.04, P=0.073); in the percentage of measured and estimated Cr clearance values that differed by greater than 20% (1-P_{20}) with respect to measured GFR (57.14 vs. 71.43, P=0.060) and RSME in measured and estimated Cr clearance values with respect to measured GFR (34.45 vs. 35.36, P=0.829).

DISCUSSION

In this study, we assessed gender differences in Cr production rate and serum Cr levels along with measured GFR and GFR markers alternative to serum Cr in ambulatory patients with cirrhosis. Our results showed a significantly lower serum Cr concentration and Cr production rate in female compared to male patients with cirrhosis (Table 2). In contrast, we did not observe significant differences in the levels of measured and estimated GFR, measured and estimated Cr clearance and non-Cr GFR biomarker levels including cystatin C, beta-trace protein, beta-2 microglobulin and dimethylarginines (Table 2). In fact, even though men had slightly better measured GFR scores than women, men had significantly higher mean serum Cr measures than women. Even after controlling for height, weight, age, race, and measured GFR, women had significantly lower serum Cr (Table 3). Our results are in line with prior studies. Lai *et al.*[9] showed that height contributed in part in gender disparity on the liver transplant waiting list; however, adjustment for height could not eliminate lower transplantation rates in women compared to men. Subsequently, similar to the findings of the study conducted by Lai *et al.*[9], we showed that adjustment for estimated liver volume or liver weight alone could not eliminate lower transplantation rates for women compared to men and contribution of the MELD score to gender disparity was greater than liver size in women.[15]

Based on our results, we propose three distinct methods can be tested in future studies to minimize underestimation of renal dysfunction in women with cirrhosis and minimize gender disparity on the liver transplant waiting list due to the use of serum Cr in the calculation of MELD-Na scores. The first and most feasible method would be replacement of serum Cr with serum cystatin C. There are several advantages to using serum cystatin C in the MELD-Na equation in lieu of serum Cr: 1) Our results showed that serum cystatin C is a gender-neutral marker of GFR among patients with cirrhosis (Table 2). 2) Our prior study showed that in patients with cirrhosis, serum cystatin C was the strongest predictor of measured GFR among other gender-neutral endogenous GFR marker alternatives to serum Cr.[24] 3) Baseline cystatin C was an independent predictor of transplant-free mortality in cirrhosis.[41, 42] Cystatin C is a suitable renal function marker in calculating the MELD score[42]. 4) Intra-individual variation of serum cystatin C was similar to serum Cr in healthy subjects.[43, 44] This suggests that cystatin C can be an alternative GFR marker for

longitudinal monitoring of renal function in cirrhosis.[44] 5) Replacement of serum Cr with serum cystatin C in the MELD-Na equation would be in line with the objectivity of the other parameters of the MELD-Na equation (i.e. total bilirubin, INR, sodium levels). Finkenstedt *et al.*[42] showed that cystatin C-based MELD had similar predictive power to MELD score. However, before the implementation of serum cystatin C in the MELD-Na equation, its intra-individual variability in cirrhosis must be evaluated in large gender- and age-controlled studies of patients with cirrhosis. Cystatin C is not without limitations. Serum cystatin C levels were shown to be affected by thyroid dysfunction[18, 45, 46] and high-dose steroids.[18, 47–49] In one report, cystatin C was associated with gender, age, height, and weight, smoking, and levels of C-reactive protein, an inflammatory biomarker independent of measured Cr clearance[22]. A major limitation of that study, however, was that measured Cr clearance was not adjusted for body surface area and this may have led to biased estimates of the association of height and weight with cystatin C[22, 50]. After controlling for measured GFR adjusted for body surface area in 103 patients with cirrhosis, our multivariable model did not show any significant association of serum cystatin C levels with age, gender, race, weight, height, C-reactive protein levels and history of hypothyroidism (Table 4).

It is certain that an ideal parameter for the replacement of serum Cr in the MELD-Na equation would be measured GFR. However, measuring GFR is laborious, costly, and impractical and more importantly requires steady-state renal function.[51, 52] As an alternative to measured GFR, serum Cr in the MELD-Na equation can be replaced with an accurate estimate of GFR. Recent studies that evaluated replacement of serum Cr by GFR estimated by Modification of Diet in Renal Disease (MDRD) equation[53] for elimination of gender disparity on the liver transplant waiting list showed conflicting results.[9, 10, 12] The MDRD equations were insufficiently accurate with respect to their ability to estimate measured GFR[34, 54–56] and perform worse in patients with cirrhosis particularly when compared to Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) Cr-cystatin C equation.[34, 39, 54] Our prior work showed serum cystatin C was an independent predictor of measured GFR in patients with cirrhosis and when used in combination with serum Cr in a novel GFR model increased the performance of the GFR model (Cr-Cystatin C GFR Equation for Cirrhosis).[24] This is likely due to the cystatin C being a Cr-blind range marker[2, 57] as the additional proportion of variance explained by adding cystatin C to the GFR model was statistically significant.[24] Indeed, the Cr-Cystatin C GFR Equation for Cirrhosis derived from our study subjects with cirrhosis[24], was validated in an independent cohort of 129 patients with decompensated cirrhosis and showed the highest accuracy for discriminating subjects with cirrhosis who had measured GFR < 60 ml/min with an area under the curve (AUC) of 0.91 compared to other GFR-estimating equations including MDRD-4[53], CKD-EPI Cr (2009)[58] and GFR-estimating equation developed from the same validation study cohort.[59]

The use of estimated Cr clearance can be considered as the last alternative to serum Cr in the MELD-Na equation to eliminate gender disparity on the liver transplant waiting list at least until cystatin C and the new Cr-cystatin C GFR Equation for Cirrhosis[24] are validated in patients with cirrhosis on the liver transplant waiting list. At first glance, this may not appear reasonable because Cr clearance underestimates renal dysfunction in cirrhosis.[3, 34] However, serum Cr as currently used in the MELD-Na equation also underestimates renal dysfunction

in patients with cirrhosis[2, 24] and additionally, it results in lower liver transplantation rates and higher mortality among women on the waiting list.[10, 12, 13] Measured Cr clearance is time-intensive and prone to collection errors[51]; further, the majority of patients with cirrhosis and high MELD-Na score on the waiting list are either oliguric or anuric. Here, we propose an alternative method for estimating Cr clearance by "dividing the gender-specific estimated Cr production rate into measured serum Cr".

Of note, this is a different estimation method than the Cockcroft-Gault equation[60] for Cr clearance. As both serum Cr and Cr production rate are significantly lower in women compared to men, both the numerator and denominator of the estimated Cr clearance calculation would account for these gender-based differences. This would make estimated Cr clearance a gender-neutral renal function measurement: *Estimated Cr clearance in ml/min/1.73m^2= (Estimated Cr production rate in mg/kg/day)*(weight in kg*100 ml)*(1.73 m^2)/(serum Cr in mg/dl)*(1440 min)*(body surface area in m^2).* The implementation of estimated Cr clearance by this method requires an equation to estimate Cr production rate. In 1978, by conducting a rigorous study among 27 patients with severe chronic renal failure, Mitch *et al.*[38] developed an equation to estimate Cr production rate.[38] We tested the performance of both measured and estimated Cr clearance using Mitch and Walser Cr production rate equation and serum Cr in reference to measured GFR. Although there was a significant bias between estimated Cr clearance and measured GFR, there was no significant difference in overall accuracy when we compared estimated Cr clearance to measured Cr clearance utilizing various metrics of overall accuracy (Table 5). This bias was likely due to the fact that Mitch and Walser equation[38] was not designed to be used in patients with cirrhosis. Therefore, we recommend that a Cr production rate equation similar to Mitch and Walser equation[38] should be developed and validated in large cohort patients with cirrhosis before Cr clearance is estimated using Cr production rate and serum Cr. In summary, we underscore our conclusion that to eliminate gender disparity on the liver transplant waiting list, use of Cr clearance should be the last alternative to serum Cr in the MELD-Na equation. Accuracy (RMSE) of both measured and estimated Cr clearances in our cohort was lower when compared to accuracy (RMSE) of Cr-Cystatin C GFR Equation for Cirrhosis (accuracy of measured Cr clearance=34.45, accuracy of estimated Cr clearance=35.36, accuracy of Cr-Cystatin C GFR Equation for Cirrhosis=22.92) (for accuracy of Cr-Cystatin C GFR Equation for Cirrhosis, see Table 3 in reference[24]).[24]

Development of a new MELD score by replacing the serum Cr in the MELD-Na equation with cystatin C, GFR estimated by Cr-Cystatin C GFR Equation or estimated Cr clearance was beyond the scope of this work. Our study was not designed to assess a cystatin C-MELD score, rather, it was specifically designed to assess gender differences in measured GFR, Cr clearance, Cr production rate and endogenous GFR biomarkers in patients with cirrhosis. Finkenstedt *et al.*[42] already developed a cystatin C-MELD score in large cohort of patients with cirrhosis and showed that gender disparity of cystatin C-MELD score was less compared to Cr-based MELD score. Our study strengthened their findings by measuring GFR and assessing gender differences in all available GFR biomarkers based on measured GFR.

We observed a significant difference in MELD-Na score classes between men and women (Table 1). More women had MELD-Na scores between 6-9; whereas more men had MELD-Na scores between 10-19 and 20-40 (Table 1). Similarly, median MELD-Na score was lower in women compared to men (12.56 vs 14.27), however this was not statistically significant. This may be related to relatively smaller number of patients with higher MELD-Na scores in our cohort as serum Cr differences between men and women become more apparent with development of sarcopenia in advanced liver disease.

The major strength of our study was that we accurately measured GFR instead of estimating it and, assessed gender disparity in cystatin C and other GFR biomarkers by controlling for measured GFR, biometrics and demographics as gender disparity due to serum Cr can be simply due to differences in renal function, biometrics and demographic characteristics between men and women.

In conclusion, our study showed that the use of serum cystatin C and other GFR biomarkers alternative to serum Cr, and also measured and estimated GFR and Cr clearance minimized between-gender biases in accounting for renal function in patients with cirrhosis.

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Disclosures:

Ayse L. Mindikoglu, M.D., M.P.H. A provisional patent application (serial no: 62/442,479) is filed with the US patent office on 01/05/2017 (Metabolomic Markers to Predict Mortality in Patients with Cirrhosis). A second provisional patent application (serial no. 62/586,966) is filed with the U.S. Patent and Trademark Office on November 16, 2017, entitled “Metabolomic Biomarkers of Hepatorenal Dysfunction and Mortality in Patients with Cirrhosis”.

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Table 1

Characteristics of 103 Subjects with Cirrhosis Stratified by Gender

		Men		Women		*P* Value
Characteristics		**N=58**	**56%**	**N=45**	**44%**	
Etiology of Cirrhosis	**Hepatitis C**	24	41	18	40	0.278
	Hepatitis B	3	5	0	0	
	Alcohol	18	31	12	27	
	Nonalcoholic Fatty Liver Disease	8	14	10	22	
	Primary Biliary Cholangitis	0	0	2	4	
	Primary Sclerosing Cholangitis	2	3	0	0	
	Autoimmune Hepatitis	1	2	2	4	
	Sarcoidosis	0	0	1	2	
	Sickle Cell Disease	1	2	0	0	
	Hemochromatosis	1	2	0	0	
Race	**Non-African-American**	46	79	31	69	0.258
	African-American	12	21	14	31	
MELD-Na Score	**6-9**	9	16	17	38	0.040
	10-19	39	67	22	49	
	20-40	10	17	6	13	
Ascites	**No Ascites**	13	22	19	42	0.014
	Diuretic-Sensitive	20	34	18	40	
	Diuretic-Refractory	25	43	8	18	
Measured GFR	**≥90 ml/min/1.73 m^2**	20	34	14	31	0.954
	≥60 and <90 ml/min/1.73m^2	20	34	15	33	
	≥30 and <60 ml/min/1.73m^2	17	29	15	33	
	≥15 and <30 ml/min/1.73m^2	1	2	1	2	
Proteinuria (> 0.5 g/24 hours)	**No**	51	93	36	92	1.000
	Yes	4	7	3	8	

Characteristics		Men N=58	Men 56%	Women N=45	Women 44%	*P* Value
	Missing	9				
Glomerular Disease (spot urine protein/Cr ratio ≥ 0.2)	**No**	47	82	31	78	0.608
	Yes	10	18	9	23	
	Missing	6				
Diabetes	**No**	41	71	32	71	1.000
	Yes	17	29	13	29	
Hypothyroidism	**No**	55	95	37	82	0.055
	Yes	3	5	8	18	
C-Reactive Protein	**≤1.0 mg/dl**	32	55	31	69	0.221
	>1.0 mg/dl	26	45	14	31	

	Men Mean	Men SD	Women Mean	Women SD	*P* Value
Age (yr)	54.40	9.50	54.71	8.02	0.860
Weight (kg)	86.76	18.09	79.69	19.99	0.066
Height (m)	1.73	0.07	1.62	0.07	<0.0001
Body-Surface Area (m^2)	2.00	0.20	1.83	0.22	0.0001
MELD-Na Score	14.27	5.24	12.56	5.31	0.109
Total Bilirubin (mg/dL)	2.81	4.90	2.03	1.61	0.263
Prothrombin Time (sec)	17.42	3.44	16.91	3.08	0.445
International Normalized Ratio	1.39	0.36	1.34	0.32	0.433
Serum Albumin (g/dL)	2.99	0.58	3.19	0.48	0.074
BUN (mg/dl)	15.90	9.76	12.60	6.60	0.046
Serum Sodium (mmol/L)	136.95	3.81	136.96	3.60	0.992

SD=Standard deviation

Table 2

Serum Cr and Endogenous GFR Biomarkers Alternative to Cr in Patients with Cirrhosis

		Men		Women		
		Mean Value	**SD**	**Mean Value**	**SD**	***P* Value**
Serum Cr	**Cr (mg/dL)**	0.97	0.37	0.82	0.28	0.023
Measured Cr Production Rate	**Measured Cr Production Rate (mg/kg/day)**	13.37	5.60	11.02	3.85	0.022
Estimated Cr Production Rate[1]	**Estimated Cr Production Rate (mg/kg/day)**	17.12	1.90	14.50	1.36	<0.0001
Measured GFR	**Measured GFR (ml/min/1.73m^2)**	81.71	35.22	78.43	34.99	0.643
Estimated GFR[2]	**Estimated GFR (ml/min/1.73m^2)**	80.73	28.05	76.27	22.86	0.393
Measured Cr Clearance	**Measured Cr Clearance (ml/min/1.73m^2)**	78.75	40.76	77.37	41.08	0.874
Estimated Cr Clearance[3]	**Estimated Cr Clearance (ml/min/1.73m^2)**	103.83	38.10	102.83	40.26	0.899
Endogenous GFR Biomarkers Alternative to Serum Cr	**Cystatin C (mg/L)**	1.18	0.50	1.16	0.38	0.811
	Beta-Trace Protein (mg/L)	1.00	0.56	0.94	0.43	0.597
	Beta-2 Microglobulin (mg/L)	4.13	2.17	3.92	1.86	0.618
	SDMA (micromole/L)	0.78	0.34	0.79	0.37	0.828
	ADMA (micromole/L)	0.69	0.21	0.62	0.14	0.076
	SDMA+ADMA (micromole/L)	1.47	0.48	1.42	0.44	0.615
	L-Arginine (micromole/L)	78.78	26.24	79.15	30.06	0.948
	L-Arginine/SDMA	115.80	57.81	116.26	56.12	0.968
	L-Arginine/ADMA	122.48	50.32	129.75	47.14	0.461
	L-Arginine/(SDMA+ADMA)	58.16	25.47	59.34	23.97	0.813

SD=Standard deviation.

[1] Mitch and Walser equation[38] was used to calculate estimated Cr production rate.

[2] Estimated GFR in ml/min/1.73m2 using Cr-Cystatin C Equation for Cirrhosis[24]=105.49 * (Cr $^{-0.712}$) * (cystatin C $^{-0.285}$) * (0.993^{age}) *(0.864^{female}) * (1.014$^{African\text{-}American}$)

[3] Estimated Cr clearance in ml/min/1.73m^2= (Estimated Cr production rate in mg/kg/day using Mitch and Walser equation[38])*(weight in kg*100 ml)*(1.73 m^2)/(serum Cr in mg/dl)*(1440 min)*(body surface area in m^2).

Table 3

Multivariate Models that included serum Cr, and GFR Biomarkers Alternative to Serum Cr controlled for Age, Race, Weight, Height and Measured GFR

Endogenous GFR Marker		Beta Coefficient for Female Gender*	Standard Error	*P* Value
Serum Cr	Cr (mg/dL)	−0.195	0.065	0.003
GFR Biomarkers Alternative to Serum Cr	Cystatin C (mg/L)	−0.130	0.094	0.169
	Beta-trace protein (mg/L)	−0.080	0.109	0.463
	Beta-2 microglobulin (mg/L)	−0.616	0.437	0.161
	SDMA (micromole/L)	−0.107	0.080	0.184
	ADMA (micromole/L)	−0.092	0.045	0.044

*To determine the association of each endogenous GFR biomarker with female gender, a separate multivariate linear regression model was developed and controlled for age, race, weight, height and measured GFR. Female gender remained associated with lower serum Cr even after controlling for age, race, weight, height and measured GFR in 103 patients with cirrhosis.

Table 4

Multivariate Analysis of Associations between Cystatin C and Demographic, Clinical and Laboratory Characteristics in 103 Patients with Cirrhosis

Variables in the Multivariate Model	Beta Coefficient	Standard Error	*P* Value
Measured GFR (ml/min/1.73m^2) *	−0.008	0.001	<0.0001
Female gender	−0.121	0.095	0.206
Age (yr)	−0.003	0.004	0.430
African-American	−0.019	0.088	0.830
Weight (kg)	0.001	0.002	0.560
Height (m)	−0.009	0.005	0.105
C-Reactive Protein (mg/dl)	0.040	0.033	0.234
History of hypothyroidism	−0.006	0.124	0.959

* In the current multivariate regression model, only measured GFR was an independent predictor of serum cystatin C.

Table 5

Bias, Precision and Accuracy of Measured and Estimated Cr Clearance in 91 Subjects with Cirrhosis

	Measured Cr Clearance	Estimated Cr Clearance[1]	*P* Value[3]
Bias[2]	1.13	−23.15	<0.0001
Precision[2]	34.43	26.72	0.066
Accuracy (1-P_{30})[2]	42.86	56.04	0.073
Accuracy (1-P_{20})[2]	57.14	71.43	0.060
Accuracy (RMSE)[2]	34.45	35.36	0.829

[1]Estimated Cr clearance in ml/min/1.73m^2= (Estimated Cr production rate in mg/kg/day using Mitch and Walser equation[38])*(weight in kg*100 ml)*(1.73 m^2)/(serum Cr in mg/dl)*(1440 min)*(body surface area in m^2).

[2]*Bias*=Mean of the difference scores (difference score=measured GFR-measured Cr clearance or estimated Cr clearance for each subject); a positive bias indicates that estimated Cr clearance on average underestimates measured GFR. *Precision=Standard* deviation of difference score. *Accuracy (1-P_{30})*=Percentage of measured or estimated Cr clearance values that differ by greater than 30%. *Accuracy (1-P_{20})*= Percentage of measured or estimated Cr clearance values that differ by greater than 20% of measured GFR. *Accuracy (RMSE)*=Root mean squared error (RMSE). Lower values for precision and accuracy (1- P_{30}), (1-P_{20}) and RMSE indicate higher precision and accuracy for measured and estimated Cr clearance.

[3]*P* values compare the performance of estimated Cr clearance to measured Cr clearance. *P* values for bias indicate difference in the location of bias, not the difference in the magnitude.