DEVELOPMENT AND VALIDATION OF A MATHEMATICAL EQUATION TO ESTIMATE GLOMERULAR FILTRATION RATE IN CIRRHOSIS: THE RFH CIRRHOSIS GFR

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Abbreviations: GFR, Glomerular Filtration Rate; MELD, Model for End-stage Liver Disease; MDRD, Modification of Diet in Renal Disease; CKD-EPI, Chronic Kidney Disease Epidemiology; IQR, interquartile range; RFH, Royal Free Hospital

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Abstract

Current expressions based on serum creatinine concentration overestimate kidney function in cirrhosis leading to significant differences between "true" and calculated glomerular filtration rate (GFR). We compared the performance of MDRD-4, MDRD-6 and CKD-EPI with "true" GFR and the impact of this difference on MELD calculation. We subsequently developed and validated a GFR equation specifically for cirrhosis and compared the performance of the new derived formula with existing GFR formulas. We included 469 consecutive patients who had a transplant assessment between 2011 and 2014. "True" GFR (mGFR) was measured using plasma isotope clearance according to a technique validated in patients with ascites. A corrected creatinine was derived from the mGFR after application of the MDRD formula. Subsequently, a corrected MELD was calculated and was compared with the conventionally calculated MELD. Stepwise multiple linear regression was used to derive a GFR equation. This was compared with the measured GFR in independent external and internal validation sets of 82 and 174 patients with cirrhosis respectively. A difference>20 ml/min/1.73m^2 between existing formulae and mGFR was observed in 226 (48.2%) patients. The corrected MELD score was ≥3 points higher in 177 (37.7%) patients. The predicted equation derived (R^2=74·6%) was: GFR=45·9x(creatinine$^{-0.836}$)x(urea$^{-0.229}$)x(INR$^{-0.113}$)x(age$^{0.129}$)x(sodium$^{0.972}$)x1·236(if male)x0·92(if moderate/severe ascites). The model was a good fit and showed the greatest accuracy compared to that of existing formulae. *Conclusion*: We developed and validated a new accurate model for GFR assessment in cirrhosis, the

RFH cirrhosis GFR, using readily available variables. This remains to be tested and incorporated in prognostic scores in patients with cirrhosis.

Kidney dysfunction is a common finding in patients with cirrhosis and is associated with increased mortality (1, 2). This is reflected by the inclusion of serum creatinine concentration in the Model for End-stage Liver Disease (MELD) score, which is a prognostic tool used for liver transplant prioritization. However, the use of creatinine for kidney function assessment in patients with cirrhosis can lead to systematic bias. Decreased creatinine production, increased tubular creatinine excretion, muscle depletion and the interference of high bilirubin levels with the analytic methods used for determination of creatinine (especially with the Jaffe reaction) may contribute to falsely low serum creatinine levels, thus leading to an overestimation of kidney function and an underestimation of liver disease severity using the MELD score (3).

Accurate measurement of GFR is particularly challenging in patients with liver disease and there is not a clear consensus as to which technique is the best reference standard. Although plasma clearance methods for assessment of GFR are recommended, because of technical difficulties, lack of availability and high cost, these are not readily available to use in routine clinical practice. Furthermore, they are prone to inaccuracy, particularly in patients with fluid retention such as ascites (4). The current BNMS Guidelines for the measurement of GFR using plasma sampling recommend not to use plasma clearance assessment of GFR in patients with ascites, oedema or other expanded body space (5). Recently, however, Wickham et al have described a modified plasma clearance method for assessment of GFR that can be used in liver patients with ascites (6, 7). For liver transplant candidates without ascites, this method showed good agreement with the “slope

intercept" technique described in the current guidelines with plasma samples taken at 2, 4 and 6 hours post injection.

The creatinine-based equations used for estimation of GFR, which did not include patients with cirrhosis when first developed, are poor predictors of kidney function in patients with cirrhosis leading to a higher than 20% overestimation of "true" GFR (8, 9).

The primary objective of this study was to develop and validate a GFR equation specifically for patients with cirrhosis, and to compare the performance of the new derived formula with the existing GFR formulae of 4-, 6-variable Modification of Diet in Renal Disease (MDRD) and Chronic Kidney Disease-Epidemiology (CKD-EPI). Secondary objectives were to assess the differences between measured and estimated GFR using common GFR formulae and between observed and "corrected" MELD score and to evaluate the predictors of these differences.

PATIENTS AND METHODS

From January 2011 to September 2014, 469 consecutive patients with cirrhosis evaluated for liver transplantation at the Royal Free Hospital were included in the study and comprised the training dataset. An independent cohort of consecutive patients with cirrhosis (n=82) with available crEDTA and cystatin measurements that were evaluated in Hippokration General Hospital of Thessaloniki in Greece was included as an external validation set. The internal validation set included 174 patients with cirrhosis assessed for a liver transplant between February 2007 and December 2010. The GFR measurement using isotope plasma clearance was part of the patients' standard pre-transplant work-up. An independent cohort of consecutive patients with cirrhosis (n=82) with available crEDTA and cystatin measurements that were evaluated in Hippokration General Hospital of Thessaloniki in Greece was included as an external validation set. Patients with simultaneous multiple organ transplantation, acute liver failure or prior liver transplantation were excluded from the analysis. The Royal Free Hospital Institutional Review Board approved the study prior to data collection. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki.

Creatinine Assessment

Creatinine concentration was measured in each sample using the O'Leary modified Jaffe method which shows the least interference with bilirubin levels (10). Creatinine measurements were measured on the Roche Analyser using Roche reagents in the Royal Free Hospital (Roche Diagnostics GmbH, Sanhofer Strasse 116, D-68305, Manheim). In the external validation

cohort, compensated Jaffe was used for creatinine measurements (Beckman Coulter CA 92821, USA) and cystatin C was analyzed by immunonephelometry using a BN-ProSpec analyzer (Dade Behring BN-ProSpec) (reference range: 0.53 to 0.95 mg/L). All creatinine measurements performed were standardized to IDMS Standards.

Glomerular Filtration Rate Assessment using radioisotope plasma clearance

For patients in the training and internal validation dataset, assessment of GFR using radioisotope plasma clearance was carried out as previously described (6, 7). For patients in the external validation set assessment of GFR was performed as recently described (11). Full details are provided in the Appendix.

Formulae for Glomerular Filtration Rate Estimation

All formulas were calculated according to published data (12-14). Details are provided in the Appendix.

Statistical Analysis

Descriptive analysis

The MDRD study equation was rearranged to give an expression for creatinine concentration in terms of GFR. This expression was used to calculate a value for corrected creatinine concentration from mGFR. Subsequently, a corrected MELD was calculated. Wilcoxon signed rank test was used to assess the differences between corrected and observed MELD scores. Full details are given in the Web Appendix.

Derivation and validation of the new equation for GFR estimation

Backward stepwise multiple linear regression on log-transformed data was used to derive a new GFR equation by using the training set. The SPSS default p values for removing or re-entering variables were used. Data were logarithmically transformed to eliminate the great variance across the range of GFR, and were subsequently re-expressed in their original units (12, 15). The following variables were considered in the univariate analysis: age, sex, ethnicity, mean arterial pressure, dry weight, height, body mass index, hand grip strength, INR, serum albumin, urea, creatinine, total bilirubin and sodium levels, presence and severity of ascites and encephalopathy, etiology of liver disease, MELD and CP scores (12, 15).

The regression coefficients determined in the training set were applied to obtain the predicted GFRs in the validation set. To determine if the new equation fits the data well, we calculated R^2 statistic, the mean difference between observed and predicted GFR (residual) values in the validation set, the root mean square error (standard deviation of the mean difference) and the appropriate residual plots.

Performance and comparison of the different equations to predict glomerular filtration rate in the training and validation set

Bias was assessed as the median difference between mGFR and estimated GFR using the new equation, MDRD and CKD-EPI study equations with negative values indicating an overestimation of mGFR (12). Precision was assessed as interquartile range (IQR) for the differences (12). Accuracy was assessed as the percentage of predictions within 10% (P10), 30% (P30)

and 50% (P50) of mGFR (8). Confidence intervals of median difference, IQR and P10, P30 and P50 were estimated with the bootstrap method (200 bootstraps). Significance testing was two-sided and set to <0·05. Analysis was performed using the SPSS statistical package (version 22.0, IBM, New York, NY, USA) and the MedCalc for Windows (version 12.5, MedCalc Software, Ostend, Belgium).

RESULTS

Demographic and clinical characteristics

The baseline patients' characteristics in the training and validation cohorts are shown in Table 1. There were significant differences in urea, albumin, sodium and mGFR levels, as well as in the prevalence of ascites and encephalopathy.

Difference between mGFR and estimated GFR

For the training dataset the median difference between estimated GFR and mGFR was 19·1 (IQR: 24·1) and 19·9 (IQR: 22·7) ml/minute/1·73 m^2 for MDRD and CKD-EPI formulae, respectively. Plots of estimated GFR versus the difference between measured GFR and estimated GFR showed a consistent overestimation of GFR when using these formulas (Figure 1a-c and Appendix Figure 1a,b).

A difference >20 ml/min/1·73 m^2 between MDRD and mGFR was observed in 226 (48.2%) patients. In multivariate binary logistic regression analysis, this difference was independently associated with male sex (odds ratio (OR): 3·5, 95% Confidence Interval (CI): 2·12-5·8), moderate/severe ascites (OR: 2·1, 95%CI: 1·31-3·38), and serum levels of sodium (OR: 0·919, 95%CI: 0·873-0·967), creatinine (OR: 0·949, 95%CI: 0·937-0·961) and bilirubin (OR: 1·003, 95%CI: 1·001-1·005). The difference was more pronounced in patients with worsening Child Pugh class (Appendix Table 1).

MELD score using "corrected" creatinine levels

The median "corrected" MELD was 14 (range 6-37) and was significantly higher than the observed median MELD score (11, range 6-61) (Wilcoxon test: z= -7·33, p<0·001). The corrected MELD score was ≥3 points higher in 177 (37·7%) patients. In the multivariate binary regression analysis, the factors significantly associated with a difference ≥3 points were: high Child-Pugh score (OR: 1·528, 95%CI: 1·367-1·708) and low creatinine levels (OR: 0·98, 95%CI: 0·97-0·988). The proportion of patients with ≥3 difference increased along with the severity of liver disease (Child-Pugh A vs. B vs. C: 19 (16·5%) vs. 88 (36·4%) vs. 70 (62·5%), respectively; p<0·001).

Prediction of glomerular filtration rate from stepwise regression analysis

The variables that were finally included in the multivariate stepwise regression model to estimate log mGFR were: log-transformed serum creatinine, urea, INR, age, sodium, bilirubin, albumin, BMI, ethnicity, sex, mild/moderate/severe encephalopathy, and moderate/severe ascites (Table 2).

The derived equation with the maximal R^2 (74·6%) was:

$$GFR = 45.9 \times (creatinine^{-0.836}\,[\frac{\mu mol}{L}]) \times (urea^{-0.229}[\frac{mmol}{L}]) \times (INR^{-0.113}) \times (age^{-0.129}[years]) \times (sodium^{0.972}[\frac{mmol}{L}]) \times 0.809\ (if\ female) \times 0.92(if\ \frac{moderate}{severe}\ ascites)\ ml/min/1.73m^2$$

We have named this equation the "Royal Free Hospital (RFH) Cirrhosis GFR".

Subsequently, this model was applied to the internal validation set to obtain predicted GFR values. The mean difference between observed and predicted GFR (residual) values in the validation set was 3·8 ml/min/1·73 m^2 with a root mean square error of 14·8. According to the residual plots the model was a good fit (Figure 2, Appendix Figure 2).

Performance of the new equation, MDRD, CG and CKD EPI formulae

Table 3 shows the performance of RFH cirrhosis GFR compared with the performances of 4-, 6-variable MDRD and CKD EPI to predict mGFR in both the external and internal validation datasets. The new equation showed the highest performance, followed by MDRD and CKD-EPI study equations. Plot analysis in the validation groups showed that the new model had greater accuracy than all others formulaes to predict mGFR (Figure 2 and 3, Appendix Figure 1d). To further evaluate the accuracy of the new equation, we calculated the percentage of the predicted GFR with the different formulae within the 10%, 30% and 50% of mGFR (P10, P30 and P50) in both cohorts. As shown in Table 3, the new equation had the highest accuracy, with P10, P30 and P50 values of 56.1%, 89% and 98.8% in the external validation and 45·4%, 88·5% and 96·6% in the internal validation cohort respectively. The performance of the new equation was not influenced by either mGFR or degree of liver dysfunction (Appendix Table 1 and 2). Although the RFH cirrhosis GFR had better overall accuracy when compared with the cystatin C based equations, this result should be interpreted with caution as the cystatin C assay was not traceable to IFCC standards and this could explain the observed overestimate.

DISCUSSION

We developed and validated the RFH Cirrhosis GFR to predict GFR in patients with cirrhosis using data from consecutive patients awaiting liver transplantation. This equation was validated in independent cohorts of patients and showed higher accuracy and less bias than the existing GFR formulae. This is of critical importance because of the shortcomings of the equations based on serum creatinine concentration which are currently used for estimating kidney function in patients with cirrhosis (16).

Levey et al (12, 15) used urinary clearance of iothalamate for reference measurements of GFR in the derivation of the Modification of Diet in Renal Disease (4-variable MDRD) and Chronic Kidney Disease Epidemiology (CKD-EPI) study equations, but Kwong et al (19) have highlighted the limitations associated with the use of this technique caused by inaccuracies in measurement of urine volumes and times, iothalamate concentration and incomplete bladder emptying and physiologic day-to-day and diurnal fluctuation in GFR. All these emphasize the importance of the new GFR equation, which is specifically developed and validated in patients with cirrhosis, and takes into consideration potential variables that affect kidney function in this setting, together with known predictors of GFR such as age and sex.

The MDRD and CKD-EPI study equations systematically overestimated kidney function and this was more pronounced in patients with worse liver function. Indicatively, the difference between MDRD and the true GFR was

greater than 20 ml/min/1·73 m^2 in approximately 50% of patients with cirrhosis. A similar discrepancy was observed using the CKD-EPI expressions (data not shown). As shown in the multivariate analysis, patients with higher bilirubin levels and lower serum sodium, and, thus, more impaired liver function, were more likely to have an overestimation of kidney function using the MDRD formula. Male sex was also independently associated with such a difference; therefore, male patients are disadvantaged using the MDRD formula, implying that the regression coefficient used for gender in the MDRD equation is inappropriate when used in cirrhosis. Presence of ascites also resulted to higher rates of overestimating true GFR; this is in line with the results by Francoz et al (9), who showed that 46% of 157 patients with cirrhosis had a GFR overestimation of ≥ 20% with the MDRD study equation. Finally, patients with lower creatinine levels are more likely to have an overestimation of GFR when using the MDRD study equation. This reflects the already discussed discrepancies between measured creatinine and renal function in cirrhosis that are more profound in patients with creatinine concentrations within the normal range (9). Therefore, a number of patients with impaired kidney function (low GFR) and, thus, at a higher mortality risk but with low serum creatinine are significantly over-scored with the existing equations for GFR calculation.

The inaccuracy of creatinine in predicting kidney function and, subsequently, mortality in cirrhosis is reflected by the large proportion of patients (40%) with ≥3 points difference between the observed and "corrected" MELD score. This has major implications in the current liver transplant allocation system, with some patients being systematically

underscored, especially those with advanced liver disease and lower measured creatinine levels. Cholongitas et al (20) showed that this difference was more profound in female candidates for liver transplantation suggesting a 3-point correction factor in females with MELD score higher than 19.

Gender and age, which are considered significant determinants of serum creatinine (21) as a result of their correlation with muscle mass, were independent predictors of GFR and were included into the new equation, similarly to existing GFR formulae. Patients with cirrhosis are commonly malnourished and/or sarcopenic (22) and, thus, the reduced muscle mass has a different impact on creatinine generation than that observed in the general population. Females have lower creatinine levels for the same GFR values compared to male patients with cirrhosis (20); therefore, gender was included in the new equation but with a different weight than the one used for GFR estimation in the other creatinine-based formulae. Urea was also an independent predictor of GFR reflecting the correlation between urea clearance and GFR; there is a difference in the ratios of the amount secreted by the tubule to the amount filtered by the glomerulus between urea and creatinine and, thus, although both are determinants of renal function, their serum levels fluctuate independently. In cirrhosis, circulating blood urea nitrogen might be increased as a result of occult gastrointestinal bleeding due to portal hypertensive gastropathy or use of steroids. Race did not have an impact on kidney function in patients with cirrhosis. Although this might be a type 2 error due to the low number of patients of black ancestry in our cohort, it seems that the weight given to race by the other creatinine-based equations is unsuitable for patients with cirrhosis compared to the general population or

to patients with chronic kidney insufficiency. On the other hand, the presence of moderate/severe ascites together with high INR values and low sodium levels had a negative correlation with GFR estimation, reflecting the impaired kidney function that accompanies patients with large-volume ascites and/or advanced liver disease (23, 24) due to the chronically reduced renal blood flow (25).

The accuracy of the new equation was satisfactory, and significantly better than the accuracy of existing formulae (89% vs. 27-75% of estimates being within 30% of true GFR respectively). The accuracy of existing formulae in our cohort is in agreement with previous reports (8) that showed that only 60-66% of estimates (using CG, MDRD-4, -5, -6 variables and the Nankivell formulae) were within 30% of the measured GFR in a large cohort of 1147 patients with cirrhosis. Precision, measured by the interquartile range for the differences, did not differ among the currently used formulae and is similar to that reported by Levey et all in the CKD-EPI prediction equation study (12).

The new equation, apart from being significantly more accurate, has several other advantages over the existing equations in patients with liver disease. Firstly, it was derived from a cohort of patients with cirrhosis at various stages of disease severity (MELD ranging from 6 to 44), including patients with hepatocellular carcinoma, and has been further validated in an independent cohort comprised of patients with diverse clinical characteristics. Secondly, it can be easily implemented in routine clinical practice as it includes readily available variables. Thirdly, it does not include variables such as albumin and bilirubin that can be influenced by several factors such as albumin infusions or require calibration in different laboratories for optimal use

(26). Lastly, it predicts GFR across a wide range of values allowing general applicability in patients with cirrhosis.

The main limitation of the new equation is the use of creatinine as the major determinant of glomerular filtration rate, which is influenced by several factors unrelated to kidney function; however, creatinine is the most readily available predictor of renal function and we tried to eliminate its weaknesses by including in the model other extra-renal determinants of renal function such age, gender and liver disease severity. The equations based on cystatin C, a protein that is eliminated almost exclusively by glomerular filtration, have better performance than the creatinine-based equations in patients with cirrhosis (27). However, the use of cystatin C is not without limitations including the high cost, its interference with several drugs such as steroids, the lack of standardization and its unsuitability in infectious conditions which are very common in end-stage liver disease (16). In an exploratory analysis, the RFH cirrhosis GFR equation performed better than cystatin C-based equations in the external validation cohort, but this needs to be further validated in larger series. Secondly, the prediction equation has been applied in a small proportion of black patients (6%) and only in the pre-transplant setting; therefore, this will need further validation in non-Caucasian populations. Finally, although the sample sizes of the training and validation cohort are relatively small compared to other validation studies of estimating equations in the general population (12, 15), it is considerably larger than previous studies evaluating kidney function in cirrhosis and the findings were quite robust (28).

In conclusion, inaccurate estimation of kidney function has major implications both in terms of prognosis but also in terms of candidate selection and prioritization for liver transplantation. We therefore developed and validated an accurate cirrhosis-specific equation for indirect GFR assessment in patients with varying disease severity, taking into consideration the most important renal and extra-renal determinants of renal function in cirrhosis. The Royal Free Hospital Cirrhosis GFR performs significantly better than existing equations such as MDRD and CKD-EPI. We strongly encourage other research teams to independently validate the performance of this equation in larger populations of patients with cirrhosis and diverse clinical characteristics. The incorporation of this cirrhosis-specific GFR equation instead of creatinine in prognostic scores such as MELD should be further tested, as it is highly likely that it will increase their overall performance.

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Figure Legends

Figure 1 Plots of estimated Glomerular Filtration Rate (eGFR) versus the difference between measured GFR (mGFR) and eGFR for a) MDRD-4, b) MDRD-6 and c) CKD-EPI in the validation dataset.

Figure 2 In the scatter/dot plot of estimated Glomerular Filtration Rate (eGFR) versus the difference between measured GFR (mGFR) and eGFR for RFH cirrhosis GFR in the internal validation plot, the residuals appear to be randomly scattered about zero. The above plots show that the model fits the data well.

Figure 3. Plot of estimated Glomerular Filtration Rate (eGFR) versus the difference between measured GFR (mGFR) and eGFR for RFH cirrhosis GFR in the external validation dataset.

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Table 1 Baseline characteristics of the study population.

		Training set (n=469)	Internal Validation set (n=174)	External Validation set (n=82)	P1 value*	P2 value**
Age (years), median (range)		55 (16-76)	54 (21-71)	53 (16-75)	0.540	0.551
Sex (M/F), n (%)		324/145 (69.1/30.9)	117/57 (67.2/32.8)	60/22 (73.2/26.8)	0.411	0.457
Black, n (%)		29 (6.2)	9 (5.2)	0	0.471	0.021
Etiology of liver disease, n (%)	Alcohol	142 (30.3)	37 (21.3)	24 (29.3)	0.137	0.073
	Viral hepatitis	135 (28.8)	56 (32.2)	36 (43.9)		
	PSC	51 (10.9)	25 (14.4)	9 (11)		
	PBC	25 (5.3)	11 (6.3)	3 (3.7)		
	NASH/Cryptogenic	54 (11.5)	13 (7.4)	10 (12.2)		
	Alcohol and Viral	4 (0.9)	15 (8.6)	0		

	Other	32 (6.8)	15 (14.1)	0		
HCC, n (%)		111 (23.7)	54 (31)	8 (9.8%)	0.070	0.005
BMI (kg/m^2), median (range)		26.9 (16.7-49.1)	26.5 (16.9-47.7)	25.6 (19.3-46.1)	0.725	0.195
BSA (m^2), mean±SD		1.9±0.3)	1.9±0.2	1.9±0.2	0.819	0.171
HGS (kg), median (range)		24 (2-47)	26.5 (9.5-56)	NA	0.060	NA
MAP (mmHg), median (range)		83 (59-122)	83.3 (63-103)	NA	0.197	NA
INR, median (range)		1.3 (0.9-5.8)	1.4 (0.9-2.8)	1.3 (1.0-7.2)	0.429	0.686
Albumin (mg/dl), median (range)		33 (16-49)	35 (18-52)	31 (16-45)	0.002	0.094
Bilirubin (µmol/L), median (range)		37 (3-639)	36 (4-932)	33.1 (7-397)	0.675	0.720
Sodium (mmol/L), median (range)		138 (113-147)	139 (120-149)	137 (117-145)	0.023	0.346
Urea (mmol/L), median (range)		5.2 (1.4-31.1)	4.8 (1.8-26.2)	5.7 (2.2-36.4)	0.029	0.132
Creatinine (µmol/L), median (range)		75 (33-464)	72 (39-261)	85.8 (43-261)	0.135	0.002
Corrected creatinine (µmol/L), median		81.06 (11.1-	NA	NA	0.251	NA

(range)		162.5)				
Measured GFR (ml/minute/1.73 m^2), median (range)		66 (7-129)	78.1 (5.7-141)	73 (16-150)	<0.001	0.048
GFR MDRD-4 (ml/minute/1.73 m^2), median (range)		87.04 (9.7-230.6)	90.9 (18.9-230.8)	76.55 (23.08-168.47)	0.359	0.001
GFR MDRD-6 (ml/minute/1.73 m^2), median (range)		81.63 (10.1-193.9)	85.2 (19.4-178.1)	NA	0.57	NA
CKD-EPI (ml/minute/1.73 m^2), median (range)		93.6 (10.3-158.7)	95.0 (20.4-156.4)	85.0 (24.0-145.0)	0.203	0.002
MELD score, median (range)		11 (6-44)	14 (6-37)	13 (5-48)	0.008	0.209
CP score, median (range)		8 (5-13)	7 (5-15)	8 (5-13)	<0.001	0.932
CP class, n (%)	A	115 (24.5)	65 (37.4)	19 (23.2)	0.004	0.932
	B	242 (51.6)	75 (43.1)	42 (51.2)		
	C	112 (23.9)	34 (19.5)	21 (25.6)		

Ascites, n (%)	No/Mild	167 (35.6)	122 (70.1)	5 (6.1)	<0.001	<0.001
	Moderate	182 (38.8)	31 (17.8)	47 (57.3)		
	Severe	120 (25.6)	21 (12.1)	30 (36.6)		
Encephalopathy, n (%)	No	388 (82.7)	140 (80.5)	64 (78.0)	0.046	0.456
	Mild/Moderate	79 (16.8)	29 (16.7)	18 (22.0)		
	Severe	2 (0.4)	5 (2.9)	0		

PSC: primary sclerosing cholangitis, PBC: primary biliary cirrhosis, NASH: non-alcoholic steatohepatitis, HCC: hepatocellular carcinoma, BMI: Body Mass Index, BSA: Body Surface Area, HGS: hand grip strength, MAP: mean arterial pressure, INR: International Normalized Ratio, GFR: Glomerular Filtration Rate, MDRD: Modification of Diet in Renal Disease, CKD-EPI: (chronic kidney disease–epidemiology), MELD: Model for End-stage Liver Disease, CP: Child-Pugh, NS: not significant, NA: not available

* Training versus internal validation group ** Training versus external validation group

Table 2 Multiple regression model to predict glomerular filtration rate on the logarithmical scale.

Variables	Coefficients	95% Confidence Interval (lower, higher)
Quantitative		
Log Creatinine (µmol/L)	-0.836	-0.920, -0.750
Log Urea (mmol/L)	-0.229	-0.293, -0.165
Log INR	-0.113	-0.200, -0.023
Log age (years)	-0.129	-0.217, -0.042
Log sodium (mmol/L)	0.972	0.320, 1.620
Qualitative		
Sex (female)	-0.092	-0.113, -0.072
Moderate/Severe ascites	-0.0369	-0.058, -0.015

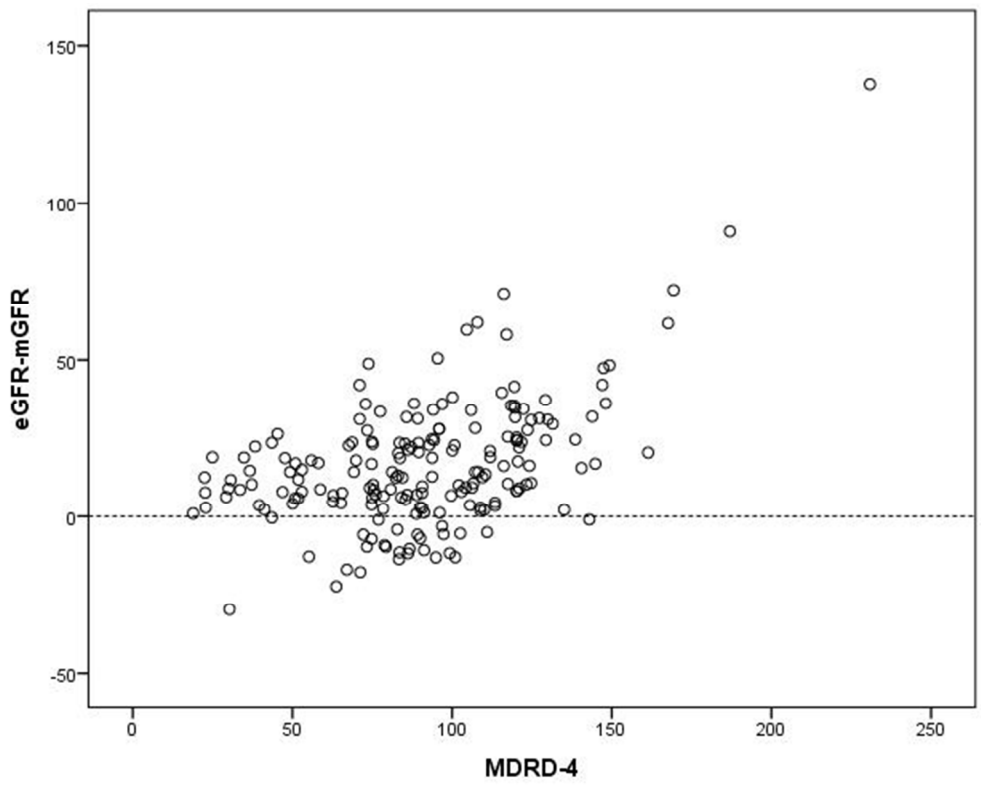
Table 3 Comparison of the performances of the new equation, MDRD and CKD-EPI equations in the training and external and internal validation cohorts.

	New equation	MDRD-4	MDRD-6	CKD-EPI	CKD-EPI cystatin C	CKD-EPI cystatin C-creatinine
	Training cohort					
Median difference (95% CI), ml/minute/1.73 m^2		-19.2 (-21.3, -17.1)	-13.0 (-15.1, -11.4)	-19.9 (-22.4, -17.9)	NA	NA
Interquartile Range for differences (95% CI), ml/minute/1.73 m^2		24.1 (21.7, 27.3)	21.3 (18.2, 23.6)	22.65 (19.9, 25.6)	NA	NA
P10 (%), (95% CI)		19 (15.3-22.4)	24.1 (20.6-28.2)	17.7 (14.5-21.1)	NA	NA
P30 (%), (95% CI)		47.8 (43.1-52.2)	60.1 (56.1-64.9)	45 (40.5-49.5)	NA	NA
P50 (%), (95% CI)		69.1 (65.3-	80.8 (77.6-	67.8 (63.5-	NA	NA

		73.3)	84.4)	72.1)		
	External validation cohort					
Median difference (95% CI), ml/minute/1.73 m^2	6.5 (4.9, 8.4)	-4.7 (-9.1, 0.5)	-22.6 (-27.4, -16.2)	-7.0 (-13.0, -2.1)	31.0 (24.4, 35.0)	16.5 (13.8, 22.0)
Interquartile Range for differences (95% CI), ml/minute/1.73 m^2	10.9 (7.9, 13.3)	23.0 (17.2, 30.2)	27.1 (22.5, 33.4)	20.0 (17.3, 27.7)	25.0 (19.0, 35.5)	17.5 (14.3, 28.1)
P10 (%), (95% CI)	56.1 (42.2-68.3)	29.3 (18.3-39.5)	19.5 (13-30)	31.7 (20.3-44.5)	9.8 (3.7-14.6)	15.9 (8.0-25.1)
P30 (%), (95% CI)	89.0 (80.7-95.7)	75.6 (66.9-86.0	46.3 (36.6-57.6)	75.6 (65.3-84.1)	26.8 (17.8-37.1)	65.9 (56.1-75.6)
P50 (%), (95% CI)	98.8 (95.1-100.0)	92.7 (86.0-97.6)	76.8 (66.7-87.9)	89.0 (80.1-95.0)	64.6 (53.7-74.9)	95.1 (89.0-98.8)
	Internal validation cohort					

Median difference (95% CI), ml/minute/1.73 m^2	3.0 (0.4, 6.4)	-14.2 (-17.6, -10.4)	-10.6 (-13.1, -9.1)	-14.9 (-19.0, -11.7)	NA	NA
Interquartile Range for differences (95% CI), ml/minute/1.73 m^2	20.4 (16.7, 24.9)	20.8 (17.6, 26.1)	19.0 (15.6, 23.2)	21.2 (17.2, 24.2)	NA	NA
P10 (%), (95% CI)	45.4 (37.0-54.0)	25.9 (19.9-32.5)	28.2 (22.6-35.1)	24.1 (17.4-31.4)	NA	NA
P30 (%), (95% CI)	88.5 (83.1-93.2)	60.9 (53.8-69.2)	70.7 (62.6-77.6)	59.2 (51.5-66.5)	NA	NA
P50 (%), (95% CI)	96.6 (93.4-98.9)	81.0 (75.0-88.0)	85.1 (79.0-89.8)	77.0 (69.9-83.6)	NA	NA

95%CI: 95% Confidence Interval, MDRD: Modification of Diet in Renal Disease, CKD-EPI: chronic kidney disease–epidemiology, NR: not relevant



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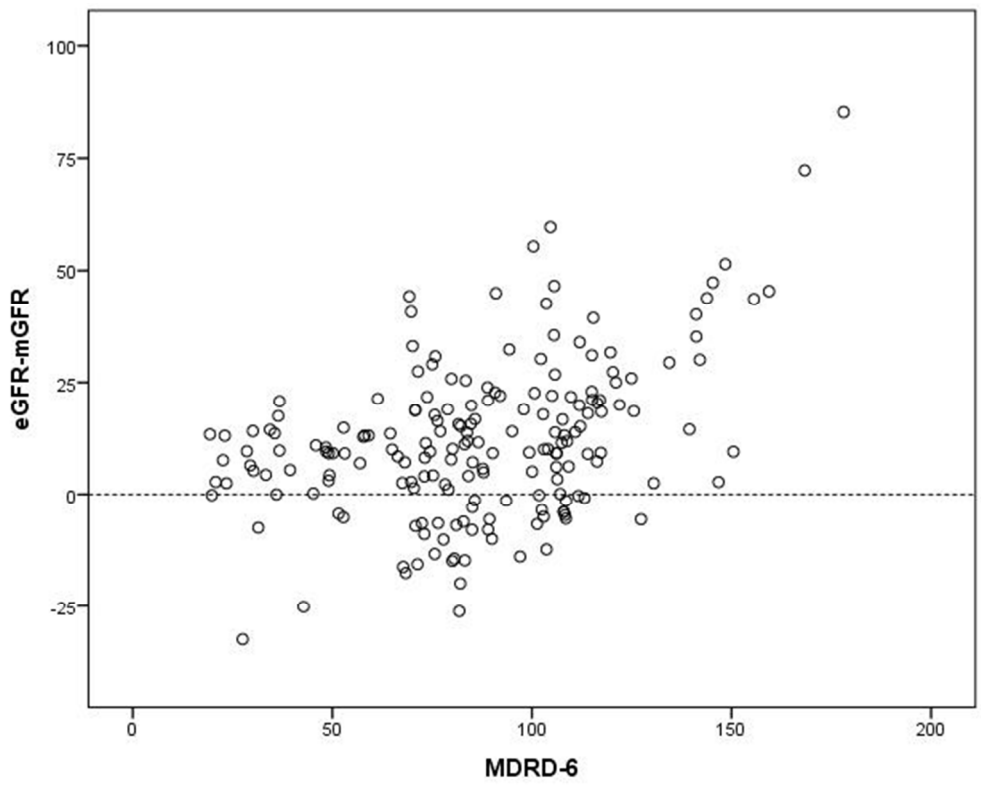

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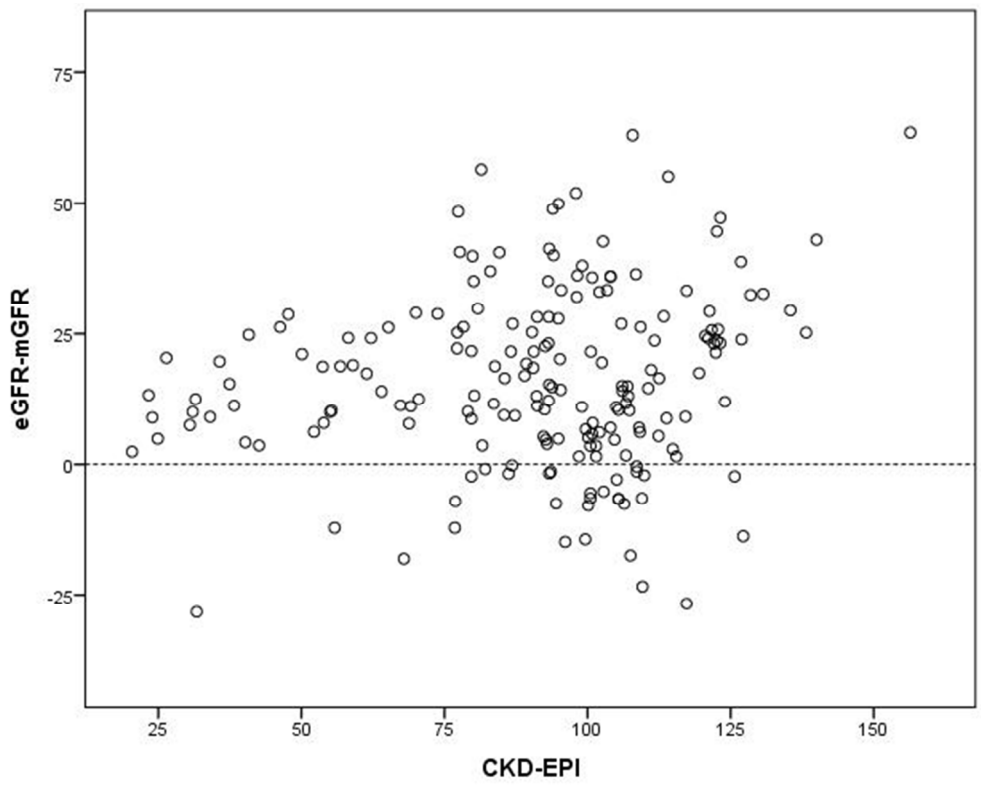

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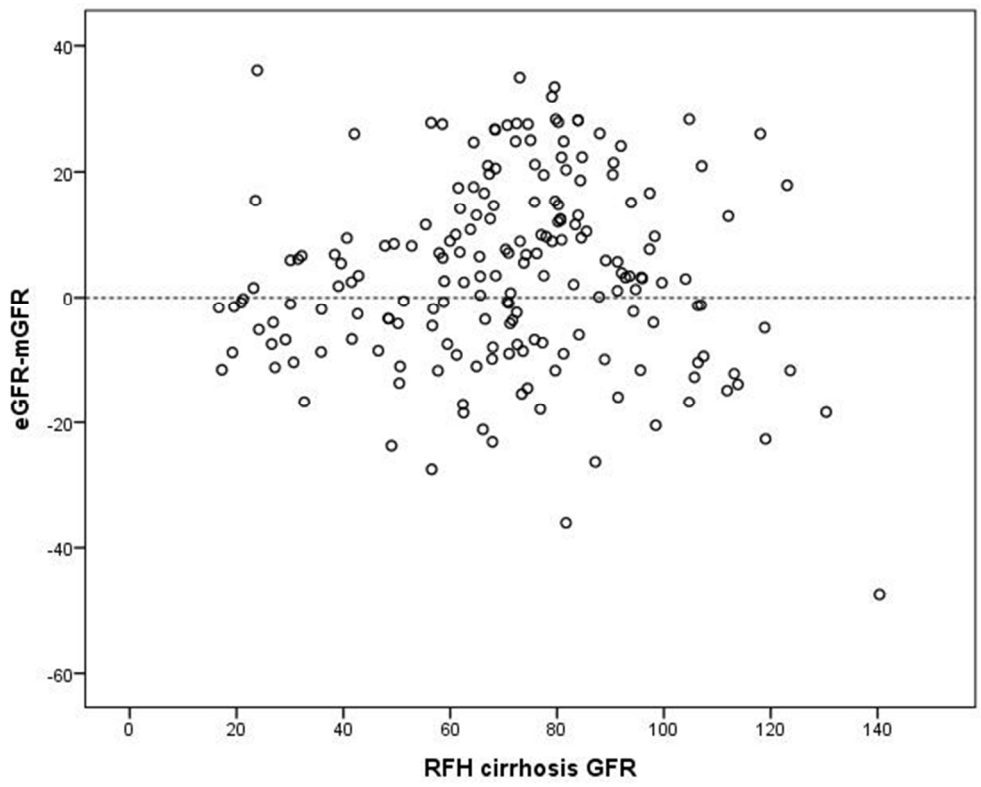

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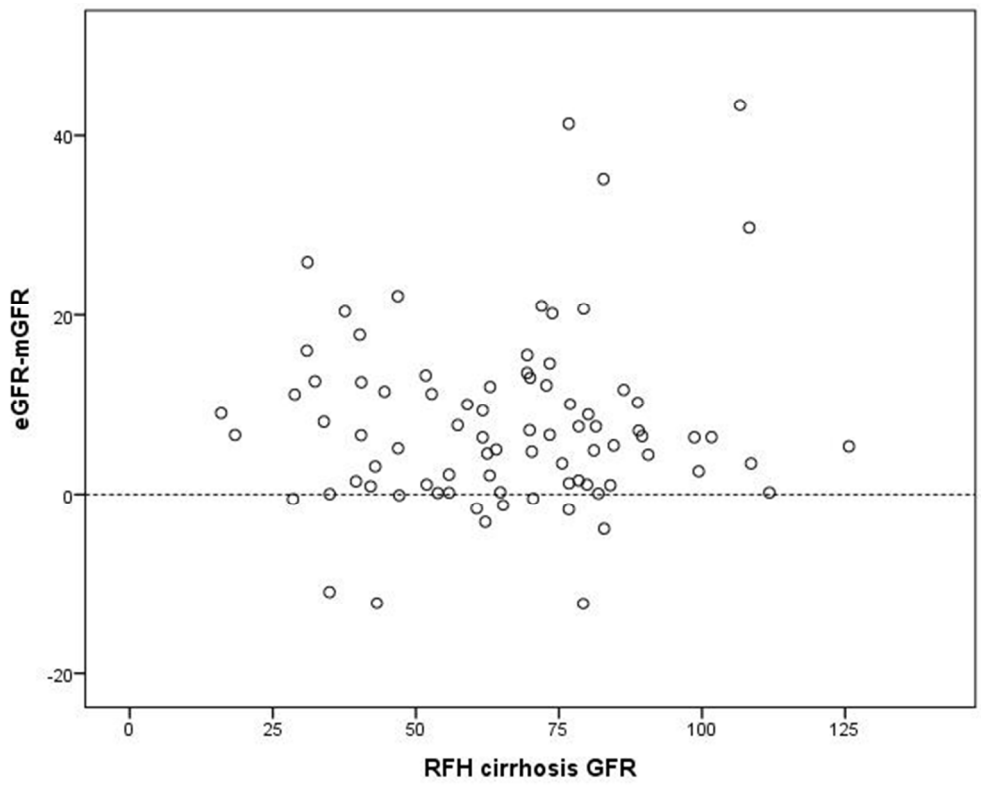

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WEB APPENDIX

Glomerular Filtration Rate Assessment using radioisotope plasma clearance

This was performed by the methods described by Wickham for the training and internal validation cohort. The "slope intercept" method described in the current BNMS Guidelines is based on the assumption that from two hours after a bolus injection of tracer the decay of plasma concentration of the tracer can be described by a single terminal exponential and so the area under the plasma clearance curve can be estimated using measurements of plasma samples taken between two and five hours post injection. The presence of ascites means that this is not the case. In order to overcome this limitation, Wickham et al have described a modified plasma clearance method for assessment of GFR that can be used in liver patients with ascites (1). In thirteen patients with known ascites the plasma clearance was fully characterized using up to 16 plasma samples taken between 5 minutes and 24 hours post injection. They showed that GFR could be calculated using plasma samples taken at 2, 4, 8, and 24 hours and using a log–linear trapezoidal rule with extrapolation to zero and infinity to calculate the area under the plasma clearance curve. In a follow-up study to validate the technique they compared this calculation with measurements based on urine collection (2). They also showed that for liver transplant candidates without ascites, this method showed good agreement with the "slope intercept" technique described in the current guidelines with plasma samples taken at 2, 4 and 6 hours post injection.

Patients were given an intravenous bolus injection of either 2·6 MBq of Cr-51 ethylenediaminetetraacetic acid (EDTA) (2007 to October 2012) or 9.5 MBq Tc-99m diethylene triamine pentaacetic acid (DTPA) (October 2012 to September 2014) in one arm. Biggi et al (3) have shown good agreement between clearance of Cr-51 EDTA and Tc-99m DTPA. Blood samples were taken from the antecubital vein contralateral to the injected arm at 2, 4, 8 and 24 hours post injection. Standard samples and plasma samples were prepared and counted according to the BNMS guidelines (4).

Clearance, CL, was calculated using Eq. (1).

$$\boldsymbol{CL} = \frac{\boldsymbol{Administered\ activity}}{\boldsymbol{AUC}}$$ (1), where AUC is the area under the plasma clearance curve given in Eq. (2).

$$AUC = \int_0^{\infty} C(t)dt$$ (2), where C(t) is the plasma concentration at time t after administration.

The AUC was calculated using a log-lin trapezoidal method. AUC of plasma concentrations between sequential time samples was estimated using exponential function interpolation. AUC before the first sample and after the last sample were calculated from exponential function back-extrapolation from the first two time samples and from forward extrapolation from the last two time samples. Further details of the AUC calculation are given in (1, 2). Calculation of the normalised GFR included a correction for a "fast exponential" as recommended in the current BNMS guidelines (4), and summarised below.

Clearance values were normalised for body surface area using Eq. (5).

$$\mathrm{CL_{norm}} = \mathrm{CL} \times \frac{1.73}{BSA}$$ (5), where $\mathrm{CL_{norm}}$ is the normalised clearance and BSA is body surface area.

The correction for the fast exponential was applied using Eq. (4).

$$\mathrm{CL_{cor-norm}} = 1.0004 \times \mathrm{CL_{norm}} - 0.00146 \times \mathrm{CL_{norm}^2}$$ (4), where $\mathrm{CL_{cor\text{-}norm}}$ is the corrected normalised clearance.

For the internal validation dataset, assessment of GFR using radioisotope plasma clearance was carried out using the "slope intercept" method described in (4) with plasma samples taken at 2, 4 and 6 hours after an intravenous bolus injection of 2·6 MBq of Cr-51 EDTA in patients without ascites. For patients with ascites the method of Wickham et al (1, 2) was used. Correction for the "fast exponential" and normalisation for BSA were carried out as described above for the training dataset.

For the external validation set, measurement of GFR was assessed with Cr-51 EDTA by sampling blood, after intravenous injection of tracer over 1-2 minutes, at 2, 4, and 6 hours. 51Chr-GFR was

calculated using the slope-intercept technique, correcting for body surface area, and the fast exponential curve recommended by the BNMS guidelines (6).

Formulae for Glomerular Filtration Rate Estimation

The simplified MDRD-4 study equation was calculated as follows: GFR (mL/minute/1·73 m^2) = 175 x (serum creatinine [mg/dl])$^{-1·154}$ x (age [year])$^{-0·203}$ x (0·762 if patient is female) x (1·21 if patient is black) (2). The extended MDRD-6 Study equation was calculated as follows: GFR (ml/minute/1·73 m^2) = 0.94086 x 170 x (serum creatinine [mg/dl])$^{-0.999}$ x (age [year])$^{-0.176}$ x (0·762 if patient is female) x (1·18 if patient is black) x (serum urea nitrogen [mg/dl])$^{-0.170}$ x (serum albumin [g/dl])$^{0.318}$ (7). GFR was normalised to body surface area (BSA) using the Dubois formula: BSA=0.007184 x $W^{0.425}$ x $H^{0.725}$ (9). The CKD-EPI equation was calculated as described by Levey et al (5). The CKD-EPI cystatin C and the CKD-EPI cystatin C-creatinine equations were calculated as described by Inker et al (10) and Stevens et al (11). The MELD score at evaluation was calculated according to the formula of the United Network for Organ Sharing available at www.unos.org (12).

Statistical Analysis

Descriptive analysis

Numerical data were expressed as mean±standard deviation if parametric or as median, interquartile range (IQR) and range (minimum to maximum) if non-parametric. All variables were tested for normal distribution using the Kolmogorov-Smirnov test. Categorical data were expressed as counts and percentages. Categorical variables were tested using the chi-square and Fisher's exact test. Continuous variables with and without normal distribution were compared using Student's t-test or the Mann-Whitney U test, respectively.

Spearman's correlation coefficient analysis was used for correlation between GFR measured with isotope plasma clearance (mGFR) and GFRs as calculated with the different formulae. Bland and Altman plots were used to assess agreement.

The MDRD study equation was rearranged to give an expression for creatinine concentration in terms of GFR. This expression was used to calculate a value for corrected creatinine concentration from mGFR. Subsequently, a corrected MELD was calculated. Wilcoxon signed rank test was used to assess the differences between corrected and observed MELD scores. Multivariate binary regression analysis (Forward LR method) was used to determine the factors predicting a difference of >20 ml/min/1·73 m2 between MDRD and mGFR, as well as a difference of ≥3 points between corrected and observed MELD.

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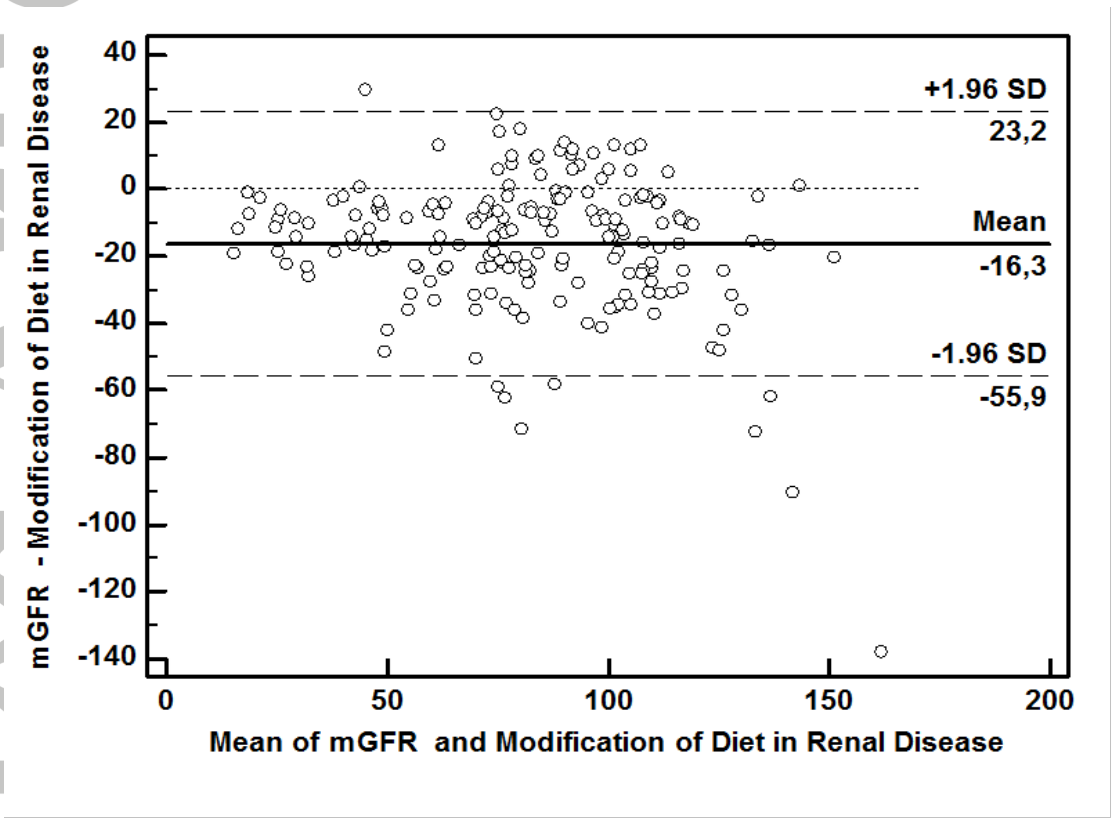
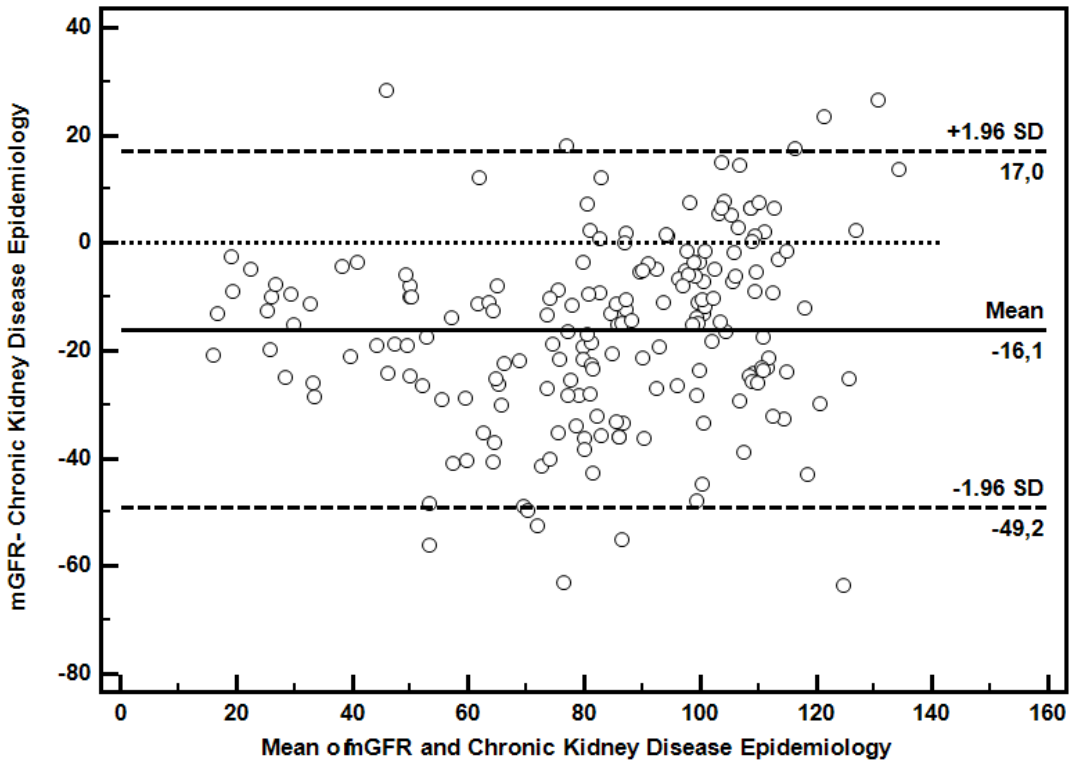
Appendix Figure 1 Bland-Altman plots comparing Glomerular Filtration Rate measured using isotope plasma clearance (mGFR) with MDRD-4 (**a**), CKD-EPI (**b**) and RFH cirrhosis GFR (**c**) formulae for GFR estimation in the validation dataset.

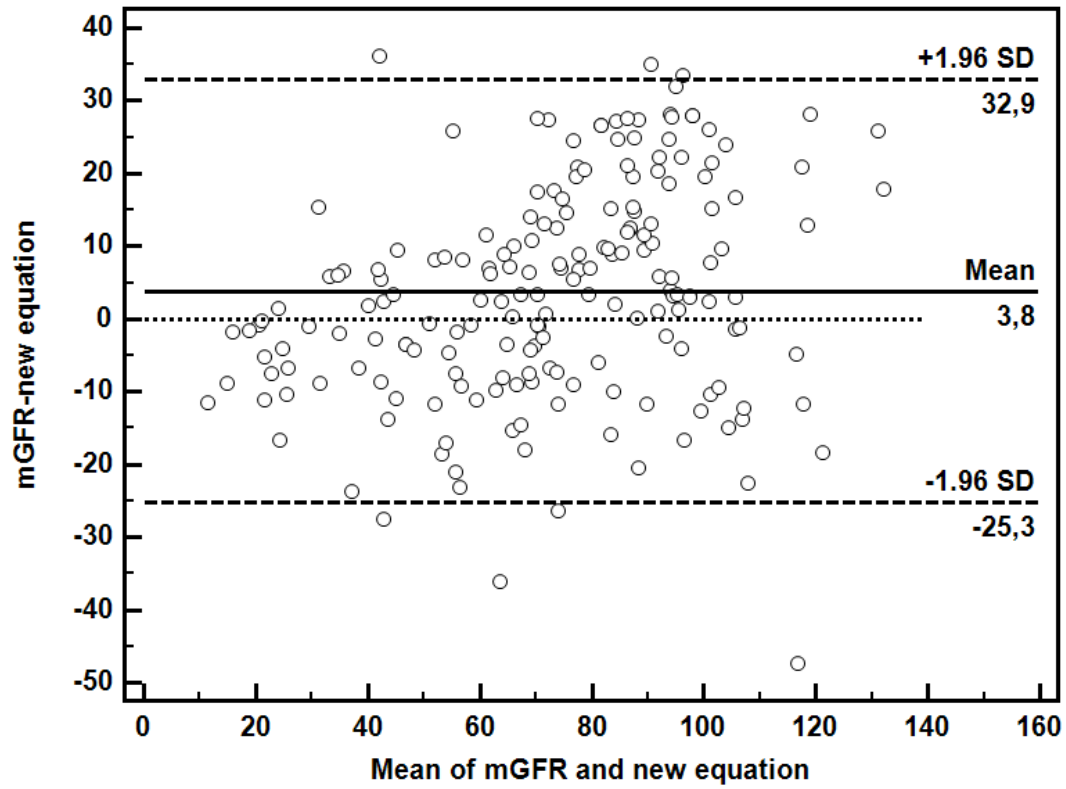
(a)



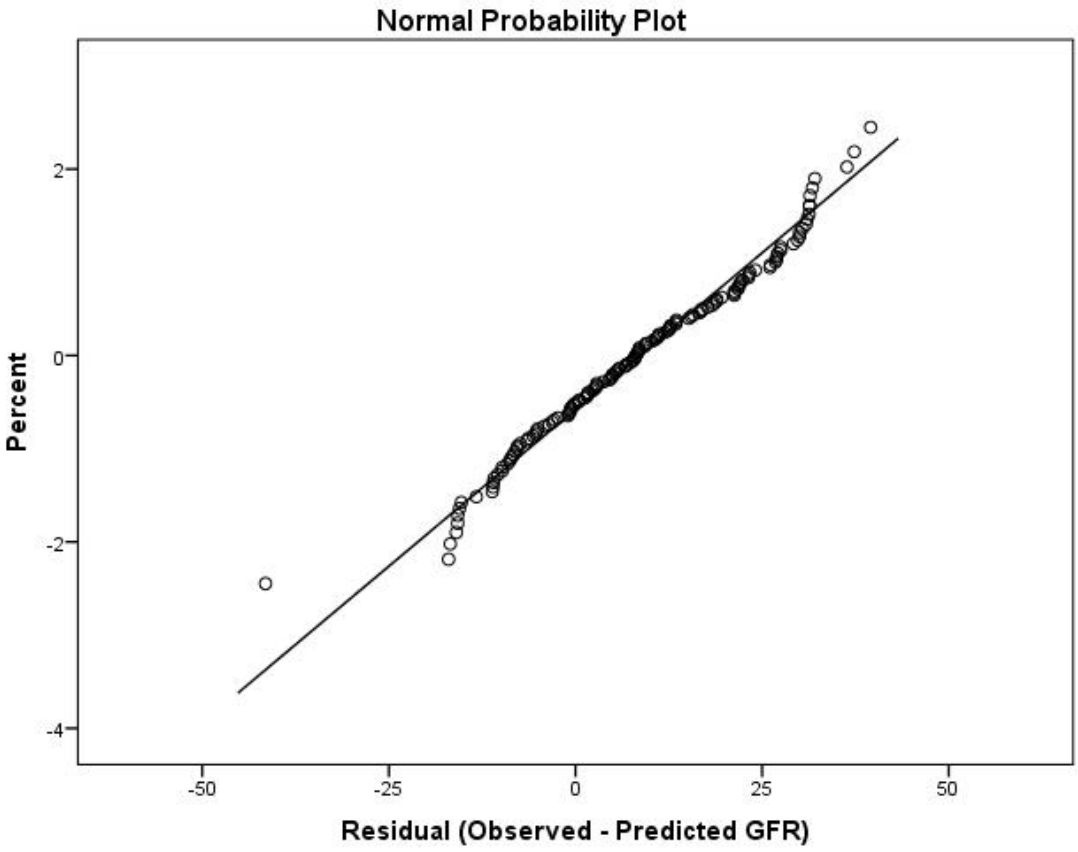
(b)



(c)



Appendix Figure 2 Normal probability plot of the difference between observed and predicted Glomerular Filtration Rate (GFR) (residuals). The residuals follow a straight line indicating that they are normally distributed.



Appendix Table 1 Performance of the new equation, MDRD-4 and CKD-EPI in both the training and the validation dataset stratified by the degree of liver failure (Child-Pugh class A versus class B versus class C).

	New equation	MDRD-4	CKD-EPI
Training cohort Child-Pugh A (n=115)			
Median difference (95% CI), ml/minute/1.73 m^2	2.2 (-1.7, 7.1)	-10.5 (-15.7, -9.0)	-13.9 (-16.7, -11.3)
Interquartile Range for differences (95% CI), ml/minute/1.73 m^2	18.5 (14.1, 25.3)	21.0 (15.9, 28.6)	19.2 (13.4, 21.3)
P10 (%), (95% CI)	50.4 (40.9-61.7)	28.7 (19.7-35.3)	25.2 (16.6-34.1)
P30 (%), (95% CI)	91.3 (83.8-96.4)	66.1 (55.3-75.9)	69.6 (60.9-78.4)
P50 (%), (95% CI)	97.4 (93.9-100.0)	87.8 (81.3-94.7)	85.2 (76.8-91.5)
Child-Pugh B (n=242)			
Median difference (95% CI), ml/minute/1.73 m^2	0.9 (-2.3, 2.5)	-19.9 (-23.3, -17.3)	-19.8 (-23.2, -17.8)
Interquartile Range for differences (95% CI), ml/minute/1.73 m^2	17.6 (15.5, 20.8)	21.7 (18.7, 26.0)	21.2 (17.3, 25.7)
P10 (%), (95% CI)	43.6 (38.6-50.1)	16.9 (13.5-22.9)	17 (12.3-22.7)
P30 (%), (95% CI)	85.5 (80.2-90.3)	47.1 (39.4-52.5)	41.9 (37.1-49.6)
P50 (%), (95% CI)	97.1 (94.4-99.2)	67.8 (61.5-73.5)	66.8 (61.1-73.7)
Child-Pugh C (n=112)			
Median difference (95% CI), ml/minute/1.73 m^2	0.05 (-2.7, 2.5)	-26.4 (-31.2, -22.5)	-28.3 (-33.7, -25.2)
Interquartile Range for differences (95% CI), ml/minute/1.73 m^2	18.5 (13.0, 22.1)	30.7 (24.2, 36.8)	27.5 (19.7, 33.3)
P10 (%), (95% CI)	40.2 (29.8-49.5)	13.4 (7.0-20.3)	11.6 (4.6-18.0)
P30 (%), (95% CI)	86.6 (78.8-94.2)	30.4 (20.9-38.6)	26.8 (18.9-34.2)
P50 (%), (95% CI)	98.2 (95.9-100.0)	52.7 (43.2-62.0)	52.7 (43.5-62.8)
Validation cohort Child-Pugh A (n=65)			
Median difference (95% CI), ml/minute/1.73 m^2	5.9 (2.6, 9.0)	-9.30 (-12.5, -6.0)	-10.4 (-14.5, -7.4)
Interquartile Range for differences (95% CI), ml/minute/1.73 m^2	19.4 (13.3, 26.4)	17.8 (10.7-22.3)	18.2 (12.9, 25.1)
P10 (%), (95% CI)	47.9 (36.4-62.8)	33.8 (22.0-47.3)	28.2 (19.6-39.8)
P30 (%), (95% CI)	94.4 (89.1-98.7)	78.9 (68.6-88.9)	77.5 (66.0-87.2)
P50 (%), (95% CI)	97.2 (93.3-100.0)	91.5 (84.3-97.9)	90.1 (83.3-96.7)

Child-Pugh B (n=75)			
Median difference (95% CI), ml/minute/1.73 m^2	5.9 (-0.3, 8.7)	-13.7 (-17.8, -8.6)	-14.1 (-21.7, -11.1)
Interquartile Range for differences (95% CI), ml/minute/1.73 m^2	25.3 (16.9, 29.4)	22.2 (16.9, 31.2)	21.7 (16.7, 32.5)
P10 (%), (95% CI)	41.5 (32.4-51.9)	26.8 (15.7-38.9)	28.0 (18.1-38.0)
P30 (%), (95% CI)	89.0 (80.6-96.1)	62.2 (52.2-72.8)	59.8 (45.9-71.5)
P50 (%), (95% CI)	95.1 (88.9-100.0)	84.1 (75.3-93.6)	79.3 (69.2-87.8)
Child-Pugh C (n=34)			
Median difference (95% CI), ml/minute/1.73 m^2	-1.7 (-7.5, 2.0)	-23.5 (-32.7, -17.5)	-26.3 (-29.1, -20.9)
Interquartile Range for differences (95% CI), ml/minute/1.73 m^2	15.04 (10.1-23.7)	23.5 (15.9, 44.3)	19.8 (10.5-32.0)
P10 (%), (95% CI)	45.9 (28.6-60.9)	10.8 (2.6-19.1)	8.1 (0.0-17.8)
P30 (%), (95% CI)	75.7 (57.9-93.5)	27.0 (12.2-43.1)	27.0 (12.0-40.8)
P50 (%), (95% CI)	94.6 (88.3-100.0)	56.8 (39.0-74.8)	45.9 (29.6-60.0)

95%CI: 95% Confidence Interval, MDRD: Modification of Diet in Renal Disease, CKD-EPI: (chronic kidney disease–epidemiology)

Appendix Table 2 Performance of the new equation, MDRD-4 and CKD-EPI in both the training and the validation dataset stratified by level of measured GFR (≥ 60 or < 60 ml/min/1.73 m^2).

	New equation	MDRD-4	CKD-EPI
Training cohort			
Measured GFR ≥60 ml/min/1.73m^2 (n=274)			
Median difference (95% CI), ml/minute/1.73 m^2	5.86 (4.00, 8.3)	-18.6 (-21.5, -16.1)	-16.7 (-19.0, -14.2)
Interquartile Range for differences (95% CI), ml/minute/1.73 m^2	20.7 (16.2, 24.2)	27.3 (23.4, 31.8)	20.2 (17.8, 24.3)
P10 (%), (95% CI)	42.7 (36.6-48.1)	26.6 (21.0-32.2)	26.3 (20.4-30.8)
P30 (%), (95% CI)	92.7 (89.7-96.1)	61.3 (55.1-67.1)	64.2 (57.9-69.7)
P50 (%), (95% CI)	99.6 (98.8-100)	81.4 (76.7-86.3)	85.4 (80.3-89.6)
Measured GFR <60 ml/min/1.73m^2 (n=195)			
Median difference (95% CI), ml/minute/1.73 m^2	-3.3 (-4.6, -2.3)	-19.7 (-22.7, -15.9)	-24.8 (-28.0, -21.2)
Interquartile Range for differences (95% CI), ml/minute/1.73 m^2	11.4 (9.0, 14.4)	21.3 (18.4, 28.4)	23.7 (18.9, 29.2)
P10 (%), (95% CI)	46.7 (40.1-54.3)	8.2 (4.2-11.3)	5.6 (2.5-9.4)
P30 (%), (95% CI)	79 (73.1-85.9)	28.7 (21.3-34.0)	17.9 (12.8-23.0)
P50 (%), (95% CI)	93.8 (90.4-97.4)	51.8 (44.9-58.8)	43 (36.5-50.5)
Validation cohort			
Measured GFR ≥60 ml/min/1.73m^2 (n=123)			
Median difference (95% CI), ml/minute/1.73 m^2	8.2 (6.2, 10.8)	-12.4 (-16.2, -9.1)	-11.7 (-14.8, -9.2)
Interquartile Range for differences (95% CI), ml/minute/1.73 m^2	20.9 (16.1, 26.1)	22.0 (18.6, 28.5)	22.6 (18.9, 27.0)
P10 (%), (95% CI)	47.4 (38.4-56.4)	32.6 (24.8-42.2)	33.3 (27.4-40.4)
P30 (%), (95% CI)	94.8 (91.4-98.2)	73.3 (65.4-81.5)	75.6 (68.4-81.3)
P50 (%), (95% CI)	99.3 (97.4-100)	93.3 (88.4-97.6)	91.9 (86.5-95.3)
Measured GFR <60 ml/min/1.73m^2 (n=51)			
Median difference (95% CI), ml/minute/1.73 m^2	-4.5 (-8.6, -1.8)	-17.0 (-22.3, -12.0)	-21.7 (-26.3, -15.7)

Interquartile Range for differences (95% CI), ml/minute/1.73 m^2	11.0 (8.3, 17.6)	19.6 (12.2, 27.5)	24.0 (14.5, 30.7)
P10 (%), (95% CI)	38.2 (23.7-54.4)	10.9 (4.1-20.3)	1.8 (0.0-6.1)
P30 (%), (95% CI)	72.7 (62.5-87.1)	32.7 (18.0-43.7)	21.8 (10.3-35.4)
P50 (%), (95% CI)	87.3 (76.8-97)	52.7 (36.7-64.6)	40.0 (24.8-52.3)

95%CI: 95% Confidence Interval, MDRD: Modification of Diet in Renal Disease, CKD-EPI: (chronic kidney disease–epidemiology)