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ORIGINAL ARTICLE

The assessment of GFR after orthotopic liver transplantation using cystatin C and creatinine-based equations

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Abstract

The measurement of kidney function after orthotopic liver transplantation (OLT) is still a clinical challenge. Cystatin C (CysC) has been proposed as a more accurate marker of renal function than serum creatinine (sCr). The aim of this study was to evaluate sCr- and CysC-based equations including the Chronic kidney disease (CKD)-EPI to determine renal function in liver transplant recipients. CysC and sCr were measured in 49 patients 24 months after OLT. The glomerular filtration rate (GFR) was calculated using the MDRD 4, the Cockroft-Gault, Hoek, Larsson, and the CKD-EPI equations based on sCr and/or CysC. As reference method, inulin clearance (IC) was estimated. Bias, precision, and accuracy of each equation were assessed and compared with respect to IC. Forty-five percent had a GFR < 60 ml/min/1.73 m^2 according to the IC. The Larsson, the Hoek and the CKD-EPI-CysC formula identified the highest percentage of patients with CKD correctly (88%, 88%, and 84%, respectively). The sCr-based equations showed less bias than CysC-based formulas with a similar precision. All CysC-based equations were superior as compared with sCr-based equations in the assessment of renal function in patients with an IC < 60 ml/min/1.73 m^2.

Introduction

Orthotopic liver transplantation (OLT) has become the therapy of choice for patients suffering from end stage liver disease [1]. Advances in surgical techniques and immunosuppressive therapy have led to an improvement in patient survival [2]. Chronic kidney disease (CKD) represents a major restraint to long-term survival after successful OLT with an incidence of 73% in the early postoperative period [3,4]. Liver recipients are at higher risk to develop CKD as compared with recipients after thoracic organ transplants, as a significant percentage of them already suffers from mild to moderate CKD before transplantation [5].

The development of CKD after OLT [3,5] is associated with poor patient survival and graft survival [6]. LaMattina et al. showed that if those factors are already present in the first year following OLT, patients are more likely to develop CKD in the long term following liver replacement [7].

Although widely used and readily available, serum creatinine (sCr) is a suboptimal marker for the definition of renal function and the development of CKD because its concentration is affected by several variables like muscle mass or distribution volume [8]. Cystatin C (CysC) is a cystein protease inhibitor that is generated in all nucleated cells at a steady state. It is generally accepted as a stable renal marker independent of gender or muscle

mass [9]. It is freely filtered at the glomerulus, reabsorbed in the proximal tubules, but not secreted in any part of the tubulus apparatus. Lately, it has been promoted as good marker for the development of CKD [10,11].

The measured glomerular filtration rate (GFR) is presently considered as the best overall index for kidney function [12]. As the GFR cannot be measured easily in clinical practice, it is regularly estimated based on equations using sCr, race, age, and body size or CysC. A four variable equation that was developed in the modification of diet in renal disease study (MDRD 4) to determine kidney function and the onset of chronic renal disease is widely accepted [13–15]. The MDRD 4 was developed in patients suffering from kidney disease and proteinuria, and a cutoff of 89 ml/min/1.73 m^2 has been defined as reduced GFR. Patients with normal GFR are often misclassified by this formula but still at risk for CKD as women are or younger and older patients [16,17]. Disease severity and underlying diseases also affect the GFR but neither factor is present in the MDRD 4 equation which limits its benefit for liver transplant recipients as well [1,6,18]. To overcome those limitations mentioned earlier, a new formula to estimate GFR has been developed by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI). The CKD-EPI equation includes sCr and reflects gender as well as race [14] and has been shown to estimate higher rates of GFR than the MDRD 4 formula and to provide a more appropriate risk classification [19–21]. To the best of our knowledge, the CKD-EPI equation has not been evaluated in a set of patients after OLT so far.

The aim of this study was to compare different GFR estimations including the new CKD-EPI equation using the inulin clearance (IC) as reference method.

Patients and methods

Study design and participants

We performed a cohort study on patients who underwent OLT at the Division of Transplantation at the Medical University of Graz between June 2008 and June 2010. The study protocol has been approved by the Research Ethics Board and informed consent was obtained from all participants. Patients >18 years of age who were undergoing OLT were eligible to participate in the study. Patients 24 months after OLT were included into the study. Pregnant women and patients with cirrhotic recurrence after OLT were excluded from the analysis. Patients with disease recurrence were excluded for their symptoms, namely ascites and developing edemas, as they might alter the results of the used standard. OLT was performed using a piggyback technique with retrograde reperfusion in all patients [22]. Demographic and clinical data were recorded for each patient by the investigators. The IC as well as sCr and serum CysC measurements were performed at 24 months after OLT in each patient. The IC was used as standard and each equation was calculated using sCr and CysC values that were measured at the same time point.

Serum CysC

Plasma CysC levels were measured routinely using an automated homogeneous immunoassay using latex or polystyrene particles coated with CysC specific antibodies based on nephelometry (particle-enhanced nepholemetric immunoassay, Dade Behring, Marburg, Germany) according to the manufacturer's instructions [23].

Serum creatinine

The sCr levels were determined using the Jaffe method using a kinetic colorimetric assay on the Roche Hitachi 917 analyzer (Roche Diagnostic GmbH, Mannheim, Germany).

Calculation of GFRs

Different equations were used to calculate the GFR in the presented study. According to the MDRD the formula $eGFR\ [MDRD4] = 186 \times sCr^{-1.154} \times Age \times [0.724$ if female patient] was calculated, according to the CKD-EPI the formula $eGFR\ [CKD\text{-}EPI] = 141 \times \min\ (sCr/k,1)^{\alpha} \times$;$\max\ (sCr/k,1)^{-1.209} \times 0.993^{Age} \times$ [1.018 if female patient] was applied. For an estimation of the GFR including CysC the Hoek [24] $eGFR = -4.32 + 80.35 \times 1/CysC$ and the Larsson [25] $eGFR = 77.239 \times CysC^{-1.2623}$ formula as well as the CysC including approach of the CKD-EPI by Stevens et al. $eGFR = 127.7 \times CysC^{-1.17} \times age^{-0.13} \times (0.91$ if female) $\times (1.06$ if black); [26]. The Cockroft-Gault formula was calculated with $eGFR = (140 - age) \times weight/sCr \times (1.23$ if male patient or 1.04 if female patient). All used equations are summarized in Table 1.

Determination of the IC

To have some reference frame for judging the utility of the different equations for renal function, IC was estimated for each study participant. Herein, we employed a single-injection technique with sufficiently long inulin serum concentration contours adapted to a two-compartment kinetic model for the determination of GFR [27]. Shortly, each study participant received an injection of 2 500 mg of sinistrin intravenously. (Sinistrin is a water-soluble form of inulin with favorable properties.) Serum concentrations of sinistrin were determined every 10 min

Table 1. Predictive equations for calculating GFR based upon sCr and serum CysC levels.

	Equations used
MDRD 4	$186 \times sCr^{-1.154} \times age \times$ [0.724 if female patient]
Cockroft-Gault	(140-age) × weight/sCr × (1.23 if male patient or 1.04 if female)
Hoeck	$-4.32 + 80.35 \times 1/sCystC$
Larsson	$77.239 \times CystC^{-1.2623}$
CKD-EPI	$141\ (sCr^{-1.154}) \times 0.993 \times (age^{-0.203}) \times$ [1.018 if female patient]
CKD-EPI-CysC	$76.7 \times CycC^{-1.19}$; eGFR = $127.7 \times sCystC^{-1.17} \times age^{-0.13} \times$ (0.91 if female patient) × (1.06 if black)

for the first 40 min after injection and every 20 min for the following 2 h thereafter. A fully enzymatic method [28] was used to measure the serum concentration of inulin. The enzymatic hydrolysis of inulin to fructose by inulinase was done in one step with the oxidation of glucose by glucoseoxidase at pH 5.2, the optimum of reaction of both enzymes. A quantity of 100 µl of sample or standard was mixed with 100 µl of hydrolysis reagent 1 (0.05 M inulinase; citrate buffer pH = 5.2; 350 U/ml inulinase (Novo Nordisk, Baegsvard, Denmark), 1200 U/ml glucoseoxidase (Sigma-Aldrich, St Louis, MO, USA) and 100 µl of hydrolysis reagent 2 (130 mM hydrogen peroxide in 0.05 M citrate buffer pH = 5.2) incubated at 56 °C for 20 min. The hydrolysate was incubated with commercially available hexokinase reagent (Roche, Mannheim, Germany) for 5 min. After taking an assay blank, phosphoglucoseisomerase was added. D-fructose-6-phosphate was converted into D-glucose-6-phosphate which reacts with NADP and glucose-6-phosphatdehydrogenase into NADPH2. The difference in the extinction at 340 nm between NADP and NADPH2 is proportional to the initial concentration of inulin. The analysis steps after hydrolysis were done using a Cobas Mira automatic analysator (Roche). The inter- and intra-assay variations of the method are 5.2% and 3.0%, respectively (500 mg/l).

Definition of OLT renal impairment

Post-transplant CKD was defined using the IC as a reference method [27,28]. Clearance results below 60 ml/min/1.73 m^2, i.e. CKD stages 3, 4 and 5 were used to define renal impairment and to summarize patients with CKD [29].

Statistical analysis

All data are presented as medians and interquartile ranges, unless otherwise stated. The Kolmogorov–Smirnov test and the Shapiro–Wilks test were used to test the normal distribution of the data. Normally distributed continuous variables were compared using the unpaired *t*-test, non normally distributed variables were compared using the Wilcoxon test. Categorical data were compared using the Chi square or the Fishers exact test. All calculated GFRs were compared at each time point to detect potential differences. A cutoff <60 ml/min/1.73 m^2 was used for all calculated GFRs to assess their detection rate of CKD at each time point. To compare the course of the different GFRs throughout the whole time period, a multivariate analysis was calculated.

To estimate the reliability of all calculated GFRs at 24 months after OLT, all estimated GFRs were compared with the respective IC values. This comparison was performed by calculating bias, precision, and accuracy as recommended in the National Kidney Foundation (NKF) guidelines on CKD [30]. Bias was defined as the mean difference between the measured IC and each estimated GFR [30]. Precision was defined as the standard deviation between the measured IC and the each estimated GFR [30]. Accuracy was defined as the percentage of GFR estimates of the different equations lying within 10 and 30% off the measured IC as defined by the NKF [30]. Bias, precision, and accuracy were calculated for the overall patient set as well as for two separate groups – patients with normal renal function according to the IC. Bland–Altman plots were calculated for all included GFRs, and ROC analysis was used to determine the sensitivity and specificity of each parameter in the assessment of renal impairment as defined by the IC. To compare different area under the receiver operating characteristics (AUROCs) a comparison according to Henley and McNeas has been performed. *P*-values smaller than 0.05 were regarded to indicate statistical significance. All data were analyzed using SPSS 15.0 (SPSS Inc., Chicago, IL, USA).

Results

Patients

We included 49 (33 male patients, 16 female patients) patients in the study. Indications for OLT were alcoholic liver disease (52%), hepatitis C (26%), hepatocellular carcinoma (10%), and other diseases (2%). Twelve percent of the included patients suffered from hepatorenal syndrome prior to OLT, 2% of those were on dialysis or received molecular absorbent renal support (MARS) therapy. Thirty percent of the patients received antihypertensive medication prior to transplantation, and 12% suffered from diabetes mellitus before OLT. All baseline characteristics of the included patients are listed in Table 2.

Table 2. Patient baseline characteristics of all included patients. No significant difference between included men and women was observed.

	Male (n = 33)	Female (n = 16)	P-value
Age (median, range)	54 (30–64)	54 (41–69)	n.s.
Indications for OLT (%)			
Alcohol	53	50	n.s.
Hepatitis	23	31	n.s.
HCC	15	0	n.s.
Others	9	19	n.s.
Hepatorenal syndrome prior OLT (%)	9	19	n.s.
Dialysis prior to OLT (%)	2.9	0	n.s.
Antihypertensive medication prior to OLT (%)	30	31	n.s.
Diabetes prior to OLT (%)	12	13	n.s.
MARS prior to OLT (%)	0	2	n.s.

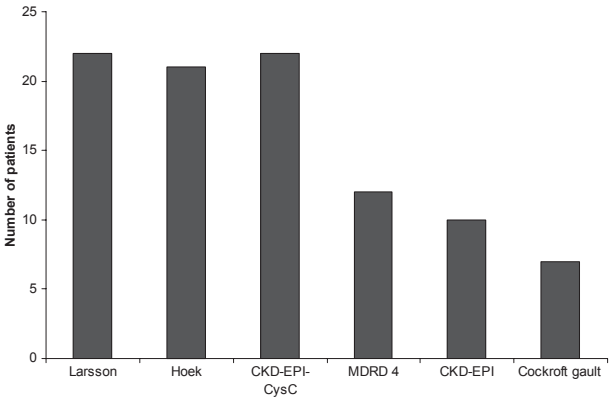

Figure 1 The IC identified 25 patients with a GFR < 60 ml/min/1.73 m^2. The Larsson, the CKD-EPI and the Hoek formula identified most of them correctly. The figure shows the number of patients that have been identified by the different equations.

Evaluation of kidney function 24 months after OLT

Kidney function after OLT was determined using the IC. On average the IC was 60.1 ml/min/1.73 m^2 in the total population and ranged from 10.9 to 97.8 ml/min/1.73 m^2. Four (8.2%) of the included patients had an IC > 90 ml/min/1.73 m^2, 20 (40.8%) patients showed IC levels between 60 and 89 ml/min/1.73 m^2 (CKD 2), 10 (20.4%) patients had IC levels between 45 and 59 ml/min/1.73 m^2 (CKD 3a), 12 (24.5%) patients showed an IC between 30 and 44 ml/min/1.73 m^2 (CKD 3b), 2 (4.1%) of the included patients showed an IC between 15 and 29 ml/min/1.73 m^2 (CKD 4) and 1 (2%) patient had an IC below 15 ml/min/1.73 m^2 (CKD 5).

Therefore, according to the IC, 25 patients suffered from CKD stages 3, 4 or 5, 24 months after liver transplantation. Among them, 48% (n = 12) were identified with renal insufficiency by the MDRD4 (OR 2.08, 95% CI: 0.9–4.6), 40% (n = 10) by the CKD-EPI (OR: 2.5; 95% CI: 1.9–5.8), 88% (n = 22) by the CKD-EPI-CysC (OR:1.2; 95% CI: 0.6–2.3), 84% (n = 21) by the Hoek formula (OR: 1.2; 95% CI: 0.6–2.5), 88% (n = 22) by the Larsson equation (OR: 1.2; 95% CI: 0.6–2.3) and 28% (n = 7) by the Cockcroft–Gault formula (OR: 4.7; 95% CI: 1.9–11.8) (Fig. 1).

Effect of immunosuppression on renal function

The administered immunosuppression comprised Sirolimus (Sir) as mTOR inhibitor and Tacrolimus (Tac) as calcineurin inhibitor (CNI) in combination with Mycophenolate Mofetil (MMF). None of the included patients received corticosteroids. Sir and Tac were administered together in 18% (n = 9) of the included patients, 47% (n = 23) of the included patients received Sir and 35% (n = 17) received Tac – both in combination to MMF. The prevalence of patients with an IC < 60 ml/min/1.73 m^2 did not differ significantly (P = 0.9) between patients who received Tac, Sir, and MMF (56%–5/9), Sir and MMF (48%–11/23), and patients who were administered Tac and MMF (53%–9/17). Therefore, the administered immunosuppressive regimen did not show any influence on the development of renal impairment after OLT.

CKD-EPI equations after OLT

The CKD-EPI equation showed a median of 69.6 ml/min/1.73 m^2 with a minimum of 22 and a maximum of 154.8 ml/min/1.73 m^2 in the overall patient set. There was a significant difference (P = 0.043) in the CKD-EPI values between men (median 76.1 ml/min/1.73 m^2, range: 22–154.8 ml/min/1.73 m^2) and women (median 59.4 ml/min/1.73 m^2, range: 23–101.4 ml/min/1.73 m^2). For patients below 60 years of age the CKD-EPI equation showed results between 42.6 and 154.8 ml/min/1.73 m^2 with a median of 76.3 ml/min/1.73 m^2 and for older recipients values between 22 and 106.7 ml/min/1.73 m^2 with a median of 60.2 ml/min/1.73 m^2 (P = 0.048).

The CKD-EPI equation, which incorporates CysC showed clearance values between 16.3 and 86.9 ml/min/1.73 m^2 with a median of 47.5 ml/min/1.73 m^2 for all patients. In patients below 60 years of age the CKD-EPI-CysC was significantly (P = 0.033) higher (mean 49.9 ml/min/1.73 m^2, 26.5–86.9 ml/min/1.73 m^2) as compared with older patients above 60 years (mean 37.9 ml/min/1.73 m^2, 16.3–53.2 ml/min/1.73 m^2). The results of the CKD-EPI-CysC formula did not differ significantly

between male patients (mean 48.2 ml/min/1.73 m^2; range: 18.7–86.9 ml/min/1.73 m^2) and female patients (mean 41.1 ml/min/1.73 m^2; range: 16.3–66.2 ml/min/1.73 m^2).

Correlation of IC and the estimated GFRs 24 months after OLT

Overall patients

Twenty-four months after liver transplantation the GFRs defined by the CKD-EPI equation ($r = 0.468$, $P = 0.002$) as well as the GFR according to the Cockroft–Gault formula ($r = 0.486$, $P = 0.001$) correlated significantly with the measured GFR defined by the IC, whereas the Hoek, the Larsson and the MDRD4 did not correlate significantly with the IC (Fig. 2).

Patients with IC < 60 ml/min/1.73 m^2

Among patients with an IC < 60 ml/min/1.73 m^2 the CKD-EPI equation ($r = 0.64$; $P = 0.001$), the Cockroft-Gault formula ($r = 0.6$; $P = 0.002$) and the CKD-EPI-CysC ($r = 0.42$; $P = 0.037$) correlated significantly with the IC. The Hoek, the Larsson and the MDRD4 did not correlate significantly with the IC.

Patients with IC > 60 ml/min/1.73 m^2

None of the included formulas showed significant correlations with the IC in patients with IC > 60 ml/min/1.73 m^2.

Bias, precision and accuracy of the different estimated GFRs

Overall patients

The CysC-based equations showed a bias of 9.7 (Hoek), 12.3 (Larsson) and 12.2 ml/min/1.73 m^2 (CKD-EPI-CysC) with precision rates of 21.4 (Hoek), 22 (Larsson) and 21.7 ml/min/1.73 m^2 (CKD-EPI-CysC). Accuracies within 10% were between 6 (Larsson) 8 (CKD-EPI-CYsC) and 14% (Hoek) and within 30% within 42 (CKD-EPI-CysC), 44 (Larsson) and 50% (Hoek), respectively. The MDRD 4 and the CG equation showed a bias of 20 and 28 ml/min/1.73 m^2, respectively, with a precision of 47.9% (MDRD 4) and 22.4% (CG) and accuracy rates within 10% of 17% (MDRD 4) and 15% (CG) and 45% (MDRD 4) and 43% (CG) within 30% (Table 3).

Patients with IC < 60 ml/min/1.73 m^2

The CysC-based equations showed accuracy rates within 10% and 30% of the IC estimates among those patients with IC < 60 with 54% (Hoek, Larsson) and 60% (CKD-EPI-CysC), respectively, for their estimates within 10% of the IC range and 74% (Larsson), 76%(Hoek) and 78%(CKD-EPI-CysC) within 30%. The CG, the CKD-EPI and the MDRD4 showed a bias of −16, −11.5, −9.5 ml/min/1.73 m^2, respectively, with precision rates of 21 (CG), 15.9 (CKD-EPI) and 12.9 ml/min/1.73 m^2 (MDRD4) and accuracies of 52% (MDRD 4), 56%

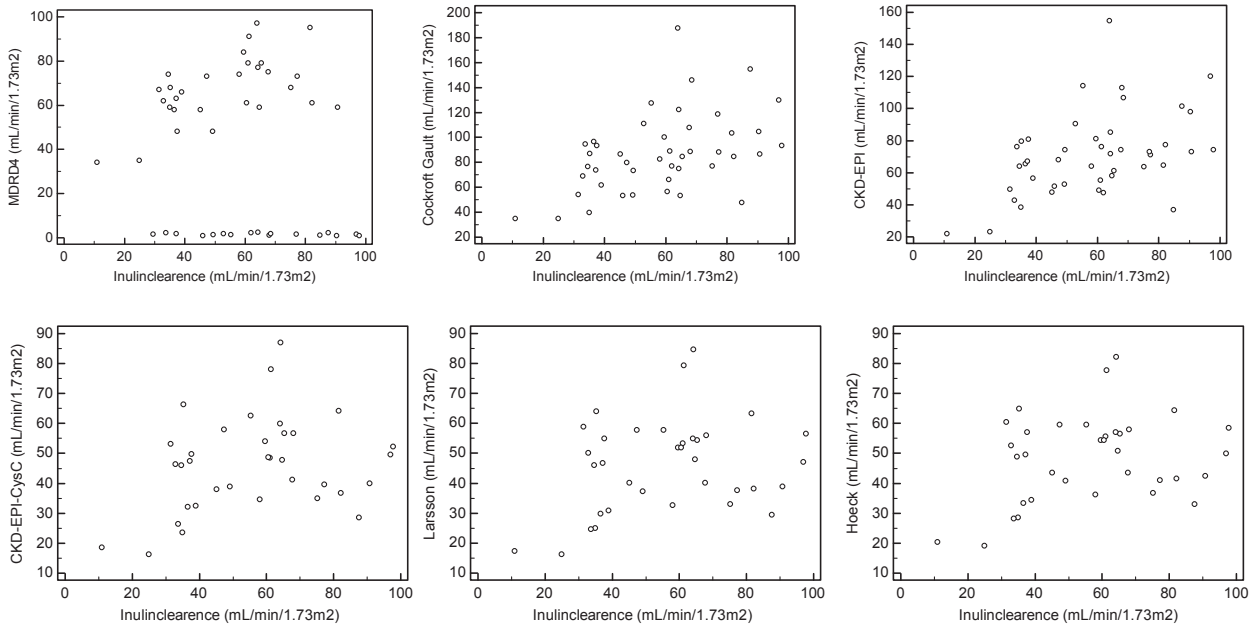

Figure 2 (a)–(f) Correlation plots comparing GFR measurements with the IC (gold standard method) in the overall patients. The CKD-EPI equation ($r = 0.468$, $P = 0.002$) as well as the GFR according to the Cockroft-Gault formula ($r = 0.486$, $P = 0.001$) correlated significantly with the measured GFR defined by the IC, whereas the Hoek, the Larsson and the MDRD4 did not correlate significantly with the IC.

Table 3. Bias, precision, and accuracy of creatinine and CysC estimates. Bias was defined as the mean difference between measured IC and estimated GFR. Precision was defined as the standard deviation of the difference between the measured IC and the estimated GFR. Both precision and accuracy are expressed as ml/min/1.73 m^2. Accuracy was defined as the proportion of values that were within 10% or 30% of the measured IC. Data were calculated for the overall patient set as well as for the subgroups according to the patient's IC levels 24 months after OLT.

	Bias	Precision	Accuracy within 10%	Accuracy within 30%
All patients				
MDRD 4 [ml/min/1.73 m^2]	20	47.9	17	45
CKD-EPI [ml/min/1.73 m^2]	−13.9	23.8	22	52
CKD-EPI-Cys C [ml/min/1.73 m^2]	12.2	21.7	8	42
Hoek [ml/min/1.73 m^2]	9.7	21.4	14	50
Larsson [ml/min/1.73 m^2]	12.3	22.0	6	44
Cockroft-Gault [ml/min/1.73 m^2]	28	22.4	15	43
IC < 60 ml/min/1.73 m^2				
MDRD 4 [ml/min/1.73 m^2]	−9.5	12.9	52	64
CKD-EPI [ml/min/1.73 m^2]	−11.5	15.9	56	52
CKD-EPI-Cys C [ml/min/1.73 m^2]	−0.3	10.1	60	78
Hoek [ml/min/1.73 m^2]	−1.6	10.4	54	76
Larsson [ml/min/1.73 m^2]	−0.3	10.8	54	74
Cockroft-Gault [ml/min/1.73 m^2]	−16	21	52	56
IC > 60 ml/min/1.73 m^2				
MDRD 4 [ml/min/1.73 m^2]	1.9	13.5	66	86
CKD-EPI [ml/min/1.73 m^2]	−2.2	18.9	64	88
CKD-EPI-Cys C [ml/min/1.73 m^2]	12.2	18.8	52	68
Hoek [ml/min/1.73 m^2]	11.1	17.3	52	72
Larsson [ml/min/1.73 m^2]	12.3	18.8	50	68
Cockroft-Gault [ml/min/1.73 m^2]	−11.3	28.9	62	76

(CKD-EPI) and 52% (CG) for 10% and of 64% (MDRD 4), 52% (CKD-EPI) and of 56% (CG) within 30% of the IC estimates (Table 3).

Patients with IC > 60 ml/min/1.73 m^2

Among those patients the bias values were −2–2 (CKD-EPI) 1.9 (MDRD4) and −11.3 ml/min/1.73 m (CG), respectively, with a precision of 18.9 (CKD-EPI), 13.5 (MDRD4), and 28.9 ml/min/1.73 m^2 (CG), respectively. Accuracies for sCr-based formulas within 10% of the IC estimates were 66% (MDRD 4), 64% (CKD-EPI), and 62% (CG), respectively, and for 30% they were 86% (MDRD 4), 88% (CKD-EPI), and 76% (CG, Table 3). The CysC-based equations showed bias (between 11.1 (Hoek), 12.2 (CKD-EPI-CysC) and 12.3 ml/min/1.73 m^2 (Larsson) with precision rates between 17.3 (Hoek), 18.8 (CKD-EPI-CysC), and 18.8 ml/min/1.73 m^2 (Larsson). Accuracies within 10% of the IC estimates were 52% (Hoek), 52% (CKD-EPI-CysC), and 50% (Larsson) and within 30% of the IC estimates accuracy was 72% (Hoek), 68% (CKD-EPI-CYsC), and 68% (Larsson, Table 3. sCr-based formulas showed significantly higher accuracies as compared with those of the formulas with CysC ($P = 0.032$) (Table 3).

Agreement between calculated GFRs and the IC

Using the Bland and Altman analysis, the Hoek, the Larsson, and the CKD-EPI-CysC equation were more accurate compared with the MDRD4, the CG and the CKD-EPI in the overall patient set. The CysC-based equations performed better than the sCr-based formulas in those patients with an IC below 60 ml/min/1.73 m^2. In the patients with an IC > 60 ml/min/1.73 m^2 the agreement of sCr and CysC-based equations did not differ significantly (Fig. 3a–f).

Influence of body mass index (BMI), age, and gender on renal function

The median BMI of the included patients was 26 (21–43), 31% ($n = 15$) of the included patients had a BMI < 25, 63% ($n = 31$) had a BMI between 25 and 30 and 6% ($n = 3$) of the included patients showed a BMI ≥ 31. The prevalence of patients with IC < 60 ml/min/1.73 m^2 did not differ significantly ($P = 0.62$) among patients with different BMI values. The median age was 57 (32–72), there was no significant ($P = 0.31$) difference between the prevalence of IC levels <60 ml/min/1.73 m^2 between patients above and below 60 years. The prevalence of an IC < 60 ml/min/1.73 m^2 did not differ significantly ($P = 0.42$) among male (48%, 16/33) and female (56%, 9/16) recipients. To assess a potential influence of age, gender, BMI, and hepatitis C infection before transplantation an AUROC analysis was performed for each formula in the above-described groups given by each parameter. The sCr- and CysC-based formulas did not differ significantly in their diagnostic accuracy between malnourished, normal, and obese patients. Among male and female patients CysC-based formulas showed higher accuracies as compared with sCr formulas in male transplant recipients ($P = 0.05$) and sCr-based equations namely the CKD-EPI and the CG formula performed better (CKD-EPI: $P = 0.05$; CG: P = n.s.) in female recipients after OLT. The sCr-based equations showed higher AUROCs as compared with CysC-based equations in patients above 60 years of age. They also performed better in those patients as compared with younger recipients. All

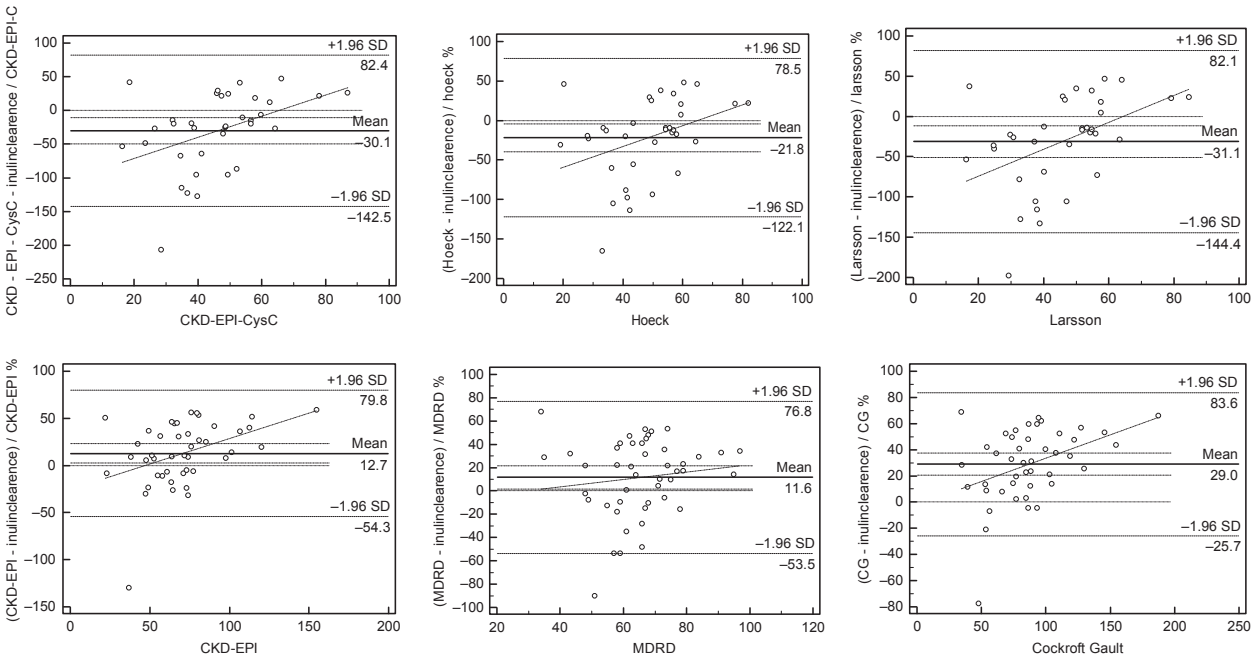

Figure 3 (a)–(f) Bland and Altman plots comparing GFR measurements with IC (gold standard method) in patients with IC < 60 ml/min/1.73 m^2 with the CysC (Hoek, Larsson, CKD-EPI-CysC) and the sCr (CKD-EPI, MDRD4, CG)-based formulas. The CysC-based formulas showed the higher agreement with the IC compared with the sCr-based equations.

equations performed better in HCV positive as compared with negative recipients ($P = 0.05$, Table 4).

Discussion

Survival after OLT has been improved significantly during the last decade. The improved survival probability increased the chance to develop CKD in the long-term follow-up after OLT, often induced by the use of calcineurin inhibitors [1,2,6]. CKD itself is a recognized risk factor and increases further morbidity and mortality [17,30,31]. As sCr-based equations tend to overestimate GFR after OLT [11,14,18,21,32,33], the only reliable tools to assess kidney function after liver transplantation are time consuming and cost expensive clearance tests to determine renal clearance rates [27,34]. The results that are derived from commonly used sCr-based formulas usually differ and do not reflect patients' kidney function accurately.

In the presented study, serum CysC-based formulas had a higher accuracy regarding the diagnosis of renal clearance rates below 60 ml/min/1.73 m^2 and showed higher correlations with the IC as a reference standard. More importantly, CysC-based equations, namely, the Larsson, the Hoek, and the CKD-EPI-CysC formula, classified more patients correctly with IC < 60 ml/min/

Table 4. To assess the influence of different patient relations, namely the nutritional state, gender, age and the HCV state a ROC analysis has been performed. The BMI, gender or age did not influence the different equations significantly, whereas all equations showed higher accuracies in HCV positive as compared with HCV negative recipients. The difference was significant especially for CysC-based equations.

	BMI			Gender		Age		HCV status	
	BMI < 25	BMI 25–30	BMI > 31	Male	Female	<60a	>60a	positive	negative
MDRD 4	0.68	0.67	0.67	0.68	0.6	0.57	0.75	0.72	0.69
CKD-EPI	0.61	0.61	0.73	0.64	0.83	0.65	0.75	0.69	0.72
Cockroft-Gault	0.64	0.64	0.77	0.68	0.73	0.66	0.75	0.79	0.53
Larsson	0.61	0.61	0.67	0.72	0.53	0.63	0.64	0.75	0.56
Hoek	0.61	0.61	0.67	0.72	0.53	0.63	0.64	0.75	0.56
CKD-EPI-CysC	0.61	0.61	0.69	0.74	0.53	0.63	0.64	0.75	0.53

1.73 m^2, as compared with those equations using only sCr. The equations using CysC also showed a higher degree of agreement with the IC (Fig. 2) than the sCr-based equations, especially in patients with IC < 60 ml/min/1.73 m^2. Formulas that incorporated CysC performed better with smaller bias and higher precision and accuracy in patients with IC < 60 ml/min/1.73 m^2, as compared with sCr-based equations. In contrast, sCr-based equations performed superior in patients with IC > 60 ml/min/1.73 m^2 (Table 3). Overall CysC-based equations were clearly superior as compared with sCr-based formulas regarding the diagnosis of CKD in patients with an IC below 60 ml/min/1.73 m^2. As those patients are already at higher risk for a progression of their renal failure this formulas are clearly of importance for the monitoring of kidney function after OLT.

The IC has already been used as reference standard to assess the prognostic value of different calculated GFRs in several patient populations including patients with liver cirrhosis before [35–37]. Therefore, we performed the IC test to accurately measure renal function of the included patients. SCr-based equations have been proven to overestimate GFR in patients after OLT [11,14,18,21,32,34]. CysC is not influenced by many of the factors that usually affect sCr in OLT recipients. Therefore, CysC-based equations were suggested to be more accurate in this patient population [38].

Various evaluations of CysC-based equations in OLT recipients and in the general population have been published so far [18,38–42]. In only a few of these, CysC-based equations were compared with measured standard clearance rates [18,38,41–43], namely the nuclear GFR and the IC. CysC has never been compared with the measured IC in OLT recipients in a controlled setting – at a defined time point – before [38,39]. Importantly, the CKD-EPI equation has never been evaluated in a cohort of liver transplant recipients so far [40,44].

In a recent study CKD-EPI gave higher estimates of GFR in subjects under the age of 70 but lower for people above 70 as compared with the MDRD 4 equation [20], which leads to an overestimation of kidney function in young people but a more accurate diagnosis in the elder population. The introduction of the CKD-EPI equation has led to a significant reclassification of patients with CKD 3a as patients with normal kidney function [21]. In a recent evaluation by Gerhardt et al. [45] the CKD-EPI equation did not differ regarding the diagnostic capacity from other sCr-based equations. The CKD-EPI equation based on sCR itself showed moderate performance in our patient cohort as well regarding the identification of patients with IC < 60 ml/min/1.73 m^2 or the evaluation of bias, precision, and accuracy in patients with IC < 60 ml/min/1.73 m^2. It overestimated the patients' renal function especially in patients with IC levels below 60 ml/min/1.73m2 (Table 3, Fig. 1). The CKD-EPI equation that incorporates CysC, detected more patients with CKD as compared with the sCR-based CKD-EPI equation and to sCr-based equations.

The CysC-based equations identified significantly more patients with an IC < 60 ml/min/1.73m^2 ($P = 0.002$) as compared with sCr-based formulas. They also showed higher accuracies, bias, and precision in patients with an IC < 60 ml/min/1.73m^2 as compared with sCr-based formulas. There are interventions available that may allow a degree of protection against progressive CKD following OLT, including the avoidance of nephrotoxic calcineurin inhibitors [31]. Thus, early detection and ongoing monitoring of those patients with a progressive loss of GFR would allow OLT recipients to benefit from interventions. However, no standard method for the accurate detection of CKD after OLT has been defined yet [6]. As CysC was able to add important information on the actual renal state in our patients it might be a good diagnostic tool to evaluate kidney function in OLT recipients on a routine basis.

Although additional investigations on the course of IC following OLT and their relation to the different prognostic GFR equations are needed, the presented study reveals that sCr-based equations alone do not seem to be sufficient for the diagnosis of CKD following OLT, and that CysC-based equations including the new CKD-EPI-CysC are able to add important information on the early detection and development of CKD after OLT.

Authorship

DW: acquired data, data analysis, wrote, and drafted the manuscript. DK: participated in research design. PS, SZ, AB, MR, MA: acquired data. GW, F-PA: research design. HM, FI, KHT: participated in data analysis. GR: made critical revisions of the manuscript. ARR: drafted the manuscript, revisions of the manuscript.

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