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ORIGINAL ARTICLE

Performance of cystatin C-based equations in a pediatric cohort at high risk of kidney injury

Edward J. Nehus · Benjamin L. Laskin · Thelma I. Kathman · John J. Bissler

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Abstract
Background Limited data exist on the performance of cystatin C-based glomerular filtration rate (GFR) equations in pediatric transplant recipients and other high-risk patients. The aim of our study was therefore to evaluate the performance of current cystatin C-based equations in this population.
Methods This was a retrospective, cross-sectional study of 141 consecutive patients (58 % post-transplant) who received a nuclear medicine GFR (NucGFR) examination using ^{99m}Tc-diethylenetriaminepentaacetic acid at our institution. Subjects included children receiving liver, kidney or hematopoietic stem cell transplants and patients with oncologic or urologic disease. GFR estimates using published GFR estimating equations, including those based on cystatin C (Filler, Zappitelli, Larsson, Hoek, Rule and Le Bricon equations, respectively) and on both cystatin C and creatinine (Zappitelli, Bouvet and Schwartz equations, respectively), were evaluated and compared to the NucGFR measurement using Bland–Altman analysis.
Results The mean NucGFR was 95 (interquartile range 76–111) ml/min/1.73 m^2. Of the cystatin C-based equations, the Rule, Hoek, Zappitelli and Schwartz (2009 CKiD equation) formulas provided the closest agreement to the NucGFR estimate. All other formulas overestimated the GFR in our cohort.
Conclusion Cystatin C-based GFR formulas can provide an accurate estimation of NucGFR in a pediatric population with a high proportion of transplant recipients and oncology patients.

E. J. Nehus · B. L. Laskin · T. I. Kathman · J. J. Bissler (✉)
Division of Nephrology and Hypertension,
Cincinnati Children's Hospital Medical Center,
3333 Burnet Avenue, MLC 7022,
Cincinnati, OH 45229-3039, USA
e-mail: john.bissler@cchmc.org

B. L. Laskin
Division of Nephrology, The Children's Hospital of Philadelphia,
Philadelphia, PA, USA

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Introduction

Accurate assessment of kidney function is essential in the care of children at high risk for renal injury and chronic kidney disease (CKD), such as those receiving a transplant. Careful attention to the glomerular filtration rate (GFR) ensures proper medication dosing and allows kidney function to be followed over time. While serum creatinine is currently used to monitor renal function, cystatin C is emerging as a potentially superior alternative [1]. Cystatin C, produced by all nucleated cells, is a 13-kDa protein inhibitor of cysteine proteases. It is freely filtered by the glomerulus without tubular secretion and is generated at a constant rate, making it an ideal marker to estimate GFR [2].

Several studies have compared cystatin C to serum creatinine for estimating GFR in children. Unlike serum creatinine, which increases with growth and development, cystatin C has been shown to be independent of age, height and body composition [3, 4]. Therefore, serum cystatin C levels parallel renal maturation, attaining peak values in the neonatal period and then decreasing during the first few months of life [5]. Despite these advantages, cystatin C has not been shown to consistently perform better than creatinine in the estimation of GFR. While some studies have suggested that cystatin C is a more accurate and sensitive marker of GFR decline [6–8], others have failed to demonstrate a significant difference [9, 10].

Many cystatin C-based equations have been published, including some which include serum creatinine and other covariates to improve GFR estimation. Most recently, Schwartz et al. reported that combining cystatin C with serum creatinine, blood urea nitrogen, height and gender provided the best estimate of GFR in 349 children in the Chronic Kidney Disease in Children (CKiD) cohort [11]. However, limited data

exist on the use of cystatin C to estimate GFR in pediatric transplant recipients and other children at high risk of renal dysfunction. The primary aim of this study was therefore to evaluate cystatin C-based formulas in children whose primary disease increased the risk of renal injury.

Patients and methods

This study was a retrospective, cross-sectional analysis of 142 consecutive children and young adults who received a nuclear medicine GFR (N5ucGFR) examination using ^{99m}Tc- diethylenetriaminepentaacetic acid (^{99m}Tc-DTPA) at Cincinnati Children's Hospital Medical Center from June to August 2010. All patients undergoing NucGFR testing for clinical indications during this time period were included. Patients were excluded if they had previously received NucGFR testing during the study period to avoid repeated measurements. Patients with more than one NucGFR examination during the study period only had their first test included in the analysis. Cystatin C values were obtained as a quality improvement effort in the Division of Nephrology clinical laboratory to evaluate the accuracy of the GFR estimation.

Examination of a residual plot of cystatin C and NucGFR revealed one significant outlier from a subject with a very high cystatin C concentration (3.34 mg/L) on the day of NucGFR testing that was inconsistent with subsequent values (0.75–1.61 mg/L). Furthermore, serum creatinine levels in this 17-year-old subject were normal, ranging from 0.6 to 0.9 mg/dL, and the NucGFR was only slightly decreased (85 ml/min/1.73 m^2). This patient was therefore excluded from the analysis, leaving a final cohort of 141 subjects.

NucGFR testing was performed in the outpatient or inpatient setting, and the NucGFR was calculated according to the methods of Balachandran et al [12]. A single dose of ^{99m}Tc-DTPA was administered with direct visualization and intermittent aspiration of blood while injecting to ensure delivery of the isotope intravenously. If extravasation was suspected, the injection site was evaluated with gamma camera imaging. The NucGFR was then computed using plasma ^{99m}Tc-DTPA disappearance curves obtained from four time points at approximately 120, 150, 180 and 210 min. Each plasma disappearance curve had a correlation coefficient of >0.98, indicating intercompartmental equilibration had occurred. A quadratic correction factor was then used to adjust the slope–intercept GFR to a two-compartment model according to the

Table 1 Glomerular filtration rate estimating equations

First author [reference]	Formula[a]	Gold standard	Population	Primary disease
Cystatin C formulas				
Filler [6]	$91.62 \times (\text{Cys C})^{-1.123}$	^{99m}Tc-DTPA	Children	CKD, kidney transplant
Zappitelli [8]	$75.94 \times (\text{Cys C})^{-1.17}$	Iothalamate	Children	CKD, kidney transplant, other transplant
Larsson [17]	$77.24 \times (\text{Cys C})^{-1.2623}$	^{99m}Tc-DTPA	Adults and children	Not specified
Hoek [16]	$-4.32 \pm (80.35/\text{Cys C})$	Iothalamate	Adults	CKD, workup for living kidney donor transplant
Rule [19]	$66.8 \times (\text{Cys C})^{-1.30}$	Iothalamate	Adults	CKD, kidney transplant, other transplant, workup for living donor transplant
Le Bricon [18]	$(78/\text{Cys C}) \pm 4$	^{51}Cr-EDTA	Adults	Kidney transplant
Cystatin C and creatinine[b] formulas				
Zappitelli [8]	$43.82 \times (\text{Cys C})^{-0.635} \times \text{SCr}^{-0.547} \times e^{0.003 \times \text{height}}$	Iothalamate	Children	CKD, kidney transplant, other transplant
Bouvet [20]	$63.2 \times (\text{SCr}^*/96)^{-0.35} \times (\text{Cys C}/1.2)^{-0.56} \times (\text{weight}/45)^{0.30} \times (\text{age}/14)^{0.40}$	^{51}Cr-EDTA	Children	Kidney transplant, drug monitoring
New CKiD [11]	$39.1 \times (\text{height}/\text{SCr})^{0.516} \times (1.8/\text{Cys C})^{0.294} \times (30/\text{BUN})^{0.169} \times (1.099)^{\text{male}} \times (\text{height}/1.4)^{0.188}$	Iohexol	Children	CKD

Cys C, Cystatin C (mg/L); SCr, serum creatinine; BUN, blood urea nitrogen (mg/dL); CKD, chronic kidney disease; ^{99m} Tc-DTPA, ^{99m} Tc-diethylenetriaminepentaacetic acid; EDTA, ethylenediaminetetraacetic acid; CKD, chronic kidney disease; CKiD, Chronic Kidney Disease in Children cohort

[a] Glomerular filtration rate (GFR) is given in units of mL/min/1.73 m^2 except in the formulas of Larsson et al. [17] and Bouvet et al. [20], where it is given in units of mL/min. SCr is given in units of mg/dL, except when indicated by a asterisk in formulas, where it is given in μmol/L

[b] The Zappitelli et al. and the CKiD studies used an enzymatic assay for creatinine measurement, whereas Bouvet et al. used a non-compensated kinetic Jaffe method

methods of Brochner–Mortensen [13], as recommended for pediatric patients by Blaufox et al. [14]. ^{99m}Tc-DTPA has previously demonstrated good agreement with inulin clearance [15]. After the GFR was measured, samples from each subject were pooled and stored at −80 °C until radioisotope decay occurred prior to the measurement of cystatin C on the pooled serum samples from each subject. Cystatin C was measured using particle-enhanced immunonephelometry (Siemens Healthcare Diagnostics, Deerfield, IL) in the Cincinnati Children's Hospital Division of Nephrology clinical laboratory.

GFR estimations from these measured serum cystatin C values were calculated according to equations published by Filler and Lepage, Zappitelli et al., Larsson et al., Hoek et al., Rule et al. and Le Bricon et al. [6, 8, 16–19]. In addition, GFR estimation from cystatin C and creatinine was performed using the equations published by Schwartz et al. (New CKiD equation), Zappitelli et al. and Bouvet et al. (Table 1) [8, 11, 20]. The Bouvet equation was corrected for body surface area.

Demographic and clinical covariates were obtained by chart review and included disease diagnosis, age, race, gender and serum creatinine. Serum creatinine values were included only if obtained on the same day of the NucGFR measurement, a criterion met by 87 subjects (62 %) from our cohort. Serum creatinine was measured locally by an isotope dilution mass spectrometry (IDMS)-traceable enzymatic assay (Fusion Chemistry Analyzer; Ortho Vitros Diagnostics, Raritan, NJ). The study was approved by the Institutional Review Board who waived the requirement for informed consent/assent as this study was a retrospective analysis of previously obtained serum samples.

All analyses were conducted using SAS statistical software (ver. 9.2; SAS Institute, Cary, NC). Descriptive statistics for continuous variables were reported as medians and interquartile ranges (IQR). For the comparisons of cystatin C-based equations to NucGFR measurements, we calculated the mean bias (mean difference between estimated GFR and NucGFR) and 95 % limits of agreement (LOA) according to the methods of Bland–Altman [21]. Sensitivity and specificity for detecting a NucGFR of <90 ml/min/1.73 m^2 (to define CKD) were also evaluated. Additionally, the proportion of the estimated (e) GFR for each formula within 10 and 30 %, respectively, of the NucGFR (accuracy) were reported, as well as the proportion of variability (R^2).

Results

Patient characteristics

A total of 141 patients were included in the analysis and their demographic and clinical characteristics are summarized in Table 2. Indications for obtaining NucGFR measurements included liver transplantation, malignancy, kidney transplantation, urologic abnormalities, hematopoietic stem cell transplantation (HSCT) and other miscellaneous conditions.

Table 2 Baseline characteristics of patient cohort

Characteristic	Patient cohort (n=141)
Age (years)	12.2 (6.1–18.5)
Gender	
Male	47 % (n=66)
Female	53 % (n=75)
Race	
Caucasian	71 % (n=100)
African–American	13 % (n=18)
Other	16 % (n=23)
Primary diagnosis	
Liver transplant	44 % (n=62)
Malignancy	26 % (n=37)
Kidney transplant	10 % (n=14)
Urologic disease	7 % (n=10)
HSCT	4 % (n=6)
Other	9 % (n=12)
Height z-score	0.03 (−0.81 to 0.72)
Weight z-score	0.17 (−0.68 to 1.11)
BMI z-score[a]	0.35 (−0.33 to 1.1)
NucGFR (ml/min/1.73 m^2)	94.9 (76.4–110.7)
Cystatin C (mg/L)	0.81 (0.69–0.97)
Creatinine (mg/dL)	0.5 (0.4–0.8)

BMI, Body mass index; HSCT, hematopoietic stem cell transplant; NucGFR, nuclear medicine glomerular filtration rate

Continuous variables are presented as the median with the interquartile range (IQR) given in parenthesis

[a] 7 patients under 2 years of age were excluded as BMI z-scores do not exist for this age range

All liver transplant recipients (n=62) were evaluated in the outpatient setting, and for 58 of these patients the post-transplant period was longer than 1 year. Only one subject showed evidence of significant liver dysfunction (increased prothrombin time and bilirubin) and was subsequently admitted 1 week after the GFR measurement for abdominal pain and ascites. Immunosuppressive medication use in the liver transplant recipients included tacrolimus (n=51), mycophenolate mofetil (n=12), cyclosporine (n=6), steroids (n=6), sirolimus (n=4) and azathioprine (n=1).

Of the 37 malignancy patients, 31 were evaluated as outpatients and six were admitted when the GFR measurement was performed. All of these six patients were admitted either after their initial diagnosis and/or for chemotherapy, except one who was admitted with fever. Seventeen patients were actively receiving or had completed chemotherapy within 2 months of the time of GFR measurement. Twenty-seven patients were diagnosed with a malignancy within 1 year of

Table 3 Comparison of cystatin C equations to nuclear medicine glomerular filtration rate (NucGFR) among different disease categories

NucGFR measurements and glomerular filtration rate (GFR) estimating equations	Disease category					
	HSCT (n=6)	Liver transplant (n=62)	Malignancy (n=37)	Urology (n=10)	Kidney transplant (n=14)	Other (n=12)
NucGFR	111.1±19.8	97.5±21.8	99.6±21.6	76.1±28.3	62.6±21.2	94.8±30.2
Cystatin C formulas (n=141)						
Filler	133.5±33.2	117.7±26.0**	135.2±35.6**	116.5±53.3**	79.1±29.1**	109.1±42.3
Zappitelli	112.5±29.2	98.7±22.6	114.1±31.3**	98.0±46.5*	65.3±24.9	91.4±36.8
Larsson	118.3±33.3	102.7±25.2*	120.2±35.6**	102.5±52.3*	66.1±26.7	94.9±41.1
Hoek	107.8±24.7	95.9±19.9	108.8±26.5*	94.3±40.4*	65.6±23.6	88.9±32.3
Rule	103.8±30.1	89.7±22.6**	104.4±32.2	89.7±47.0	57.0±23.5[a]	82.8±36.9
Le Bricon	112.8±24.0	101.3±19.3	113.9±25.7**	99.7±39.2**	71.9±22.9*	94.5±31.3

Data are reported as the mean ± standard deviation (in mL/min/1.73 m^2, except for the Larsson formula, where it is given in mL/min)

*, **GFR estimate is significantly different from the NucGFR measurement at $p<0.05$ and $p<0.01$, respectively

[a] Rule et al. developed a separate equation for kidney transplant recipients ($GFR=(76.6*cys\ C)^{-1.16}$). Using this formula, mean GFR was 65.9± 24.9, which was not significantly different from the NucGFR measurement

the GFR measurement, while ten were over 1 year removed from the initial diagnosis.

All kidney transplant recipients were evaluated as outpatients, and all but one were over 1 year post-transplant. Immunosuppressive medication use included sirolimus (n=8), tacrolimus (n=5), cyclosporine (n=2), mycophenolate mofetil (n=10), steroids (n=5) and azathioprine (n=3). Five of six HSCT recipients received autologous stem cell transplants for neuroblastoma. Three patients were evaluated as outpatients and three as inpatients, two of whom had received chemotherapy for relapsed disease.

The remainder of our cohort was comprised of ten patients with urologic disease (six with cloacal anomalies or anorectal malformations) and 12 patients with other diseases (nine with underlying hematologic disease and undergoing evaluation prior to HSCT). Only three of these remaining patients were hospitalized at the time of GFR measurement.

Comparison of cystatin C-based equations in different disease categories

Cystatin C-based equations were compared to NucGFR measurements in patients with different underlying diseases (Table 3). Of previously published equations, the Rule formula performed best, accurately estimating GFR in all patients except liver transplant recipients, in whom there was about an 8 ml/min/1.73 m^2 underestimation of GFR. The Zappitelli, Larsson, Hoek, and Le Bricon equations also performed reasonably well, although the GFR was significantly overestimated in urologic and oncology patients. The Filler equation consistently overestimated renal function in all patient categories. Among those equations including cystatin C and creatinine (Table 4), the New CKiD formula significantly outperformed the Bouvet and Zappitelli equations, which overestimated GFR in most patient categories.

Table 4 Comparison of cystatin C and creatinine estimating equations to nuclear medicine glomerular filtration rate (NucGFR) among different disease categories

NucGFR measurements and glomerular filtration rate (GFR) estimating equations	Disease category (n=87)					
	HSCT (n=4)	Liver transplant (n=47)	Malignancy (n=15)	Urology (n=4)	Kidney transplant (n=13)	Other (n=4)
NucGFR	118.2±20.2	98.6±21.0	104.0±22.8	77.9±26.2	64.3±20.9	76.0±40.7
Cystatin C and creatinine formulas						
Zappitelli	163.8±33.3*	111.4±26.1**	132.6±33.4**	89.4±31.3	64.8±25.5	101.6±54.2*
Bouvet	169.2±27.2**	123.6±24.3**	135.7±32.3**	109.4±26.9*	83.4±29.7**	116.0±60.2*
New CKiD	126.4±28.2	100.3±19.2	115.8±27.9	74.7±25.7	67.3±23.0	88.3±38.0

Data are reported as the mean ± SD (in mL/min/1.73 m^2, except for the Bouvet formula, where it is given in mL/min)

*, **GFR estimate is significantly different from the NucGFR measurement at $p<0.05$ and at $p<0.01$, respectively

CKiD, chronic kidney disease in children

Comparison of cystatin C-based equations and creatinine-based equations in the entire cohort

The performance of each formula based on mean bias, 95 % LOA, accuracy, sensitivity and specificity for estimating NucGFR and predicting renal insufficiency (NucGFR <90 ml/min/1.73 m^2) in the entire cohort is summarized in Table 5. The only established formulas that did not have a significant mean bias were the Rule and Hoek formulas. The Zappitelli (including only cystatin C) and New CKiD equations also performed well, with mean biases of only 5.9 and 4.2 ml/min/1.73 m^2, respectively. All other formulas more significantly overestimated GFR, especially the Filler equation and creatinine-based GFR formulas derived by Bouvet and Zappitelli (mean biases ranging from 15.8 to 27.5 ml/min/1.73 m^2).

Discussion

We evaluated the accuracy and performance of cystatin C equations in a pediatric cohort at high risk of renal injury. The majority of our subjects did not have significant underlying renal disease at the time of assessment, as evidenced by the cohort median GFR of >90 ml/min/1.73 m^2. We found that the Rule, Hoek, Zappitelli and New CKiD equations provided reasonably accurate assessments of GFR in our patients. In contrast, the Filler equation and the other creatinine-based equations (Bouvet and Zappitelli combined formula) significantly overestimated the GFR in our subjects.

The limitations of using only creatinine to estimate GFR are well documented and include variability due to gender, age, tubular secretion and muscle mass [22]. To improve the estimation of kidney function, other investigators have developed cystatin C-based equations in children and adults with known kidney disease [6, 8, 11, 16–20]. Many of these adult studies have rigorously assessed GFR estimating equations in liver and kidney transplant recipients, as well as patients with malignancy (Table 6). However, the formulas published to date have not been analyzed or validated in a large group of pediatric patients with similar high-risk conditions.

In the pediatric liver transplant population, Samyn et al. measured renal function in 62 children but did not report cystatin C-based GFR estimation [23]. Berding et al. reported on 48 pediatric liver transplant recipients and found reasonable performance of cystatin C, but assessed only the Filler equation [24]. Several additional pediatric studies have included a variable proportion of transplant recipients, including those of Grubb et al. [7] (unclear how many transplant patients), Filler et al. [6] (only 5.4 % received a kidney transplant), Bouvet et al. [20] (48 % of cohort were kidney transplant recipients) and Zappitelli et al. [8] (27 % kidney transplant, 7 % 'other' transplants). Finally, we identified one pediatric study comparing the Filler cystatin C equation to the new Schwartz formula in 68 children with malignancy [25].

Recently, Bacchetta et al. published a well-designed analysis validating cystatin C-based equations in 252 children at high risk of renal injury [26]. However, in contrast to our cohort, most of these children had primary renal disease, and only 26 subjects (10 %) received a non-renal organ transplant. The authors concluded that the Le Bricon, Larsson, Rule and both Zappitelli formulas provided the most accurate agreement with inulin clearance, whereas the Filler equation overestimated GFR and the Bouvet formula underestimated GFR. Similarly, in our study, the Rule and Zappitelli equations performed reasonably well, although the Larsson and Le Bricon formulas significantly

Table 5 Overall performance of glomerular filtration rate (GFR) estimating equations

Formula	Mean Bias (95 % confidence interval)	95 % limits of agreement	Sensitivity[a] (%)	Specificity[a] (%)	R^2	30 % accuracy (%)	10 % accuracy (%)
Cystatin C formulas (n=141)							
Filler	24.9 (20.8–29.0)	−23.2 to 72.9	49.1	96.4	0.54	56.0	19.9
Zappitelli	5.9 (2.3–9.5)	−36.5 to 48.2	68.4	88.1	0.54	80.9	36.2
Larsson	10.2 (6.2–14.2)	−37.3 to 57.7	66.7	89.3	0.53	73.8	34.8
Hoek	2.7 (−0.5–5.9)	−34.9 to 40.3	71.9	84.5	0.55	85.8	36.2
Rule	−2.8 (−6.5–0.8)	−46.0 to 40.3	80.7	66.7	0.53	84.4	36.9
Le Bricon	8.4 (4.9–11.2)	−28.9 to 45.0	64.9	90.5	0.55	77.3	37.6
Cystatin C and creatinine formulas (n=87)							
Bouvet	27.5 (23.2–31.8)	−12.1 to 67.1	31.6	100	0.65	49.4	21.8
Zappitelli	15.8 (11.3–20.2)	−25.2 to 56.7	60.5	93.9	0.70	70.1	24.1
New CKiD	4.2 (0.3–8.2)	−32.3 to 40.8	68.4	81.6	0.58	86.2	43.7

[a] Sensitivity and specificity to detect a GFR of <90 ml/min/1.73 m^2

CKiD, chronic kidney disease in children

Table 6 Summary of literature of cystatin C-based estimating equations in transplant and other high-risk patients

Study	Age group	*n*	Formulas assessed	GFR mean ± SD (gold standard)	Best bias (mean difference)	Best precision (SD difference[a])	Best 30 % accuracy (%)	Best 10 % accuracy (%)
Liver transplant								
Gerhardt [34]	Adult	59	MDRD, Filler, Hoek, Larsson	52.3±17.5 (DTPA)	Hoek (−0.1) median	Hoek (15.7)	Hoek (76.3)	
Boudville [32]	Adult	41	MDRD, CG, Hoek, Larsson, Filler, Le Bricon	58±19.8 (EDTA)	Le Bricon (3.4)	MDRD (12.2)	Le Bricon/MDRD (80)	Le Bricon (27)
Ling [38]	Adult	60	Hoek, Filler, Larsson, Own	96.8±32.8 (DTPA)	Own (0.6)	Own (8.5)	Own (77)	Own (30)
Berding [24]	Child	48	Schwartz, Filler	62±20 (EDTA)	Filler (6)			
Kidney transplant								
Risch[b] [39]	Adult	29	Larsson, MDRD, Own	45 (iothalamate)	MDRD (1.7)		MDRD (72)	
Qutb [40]	Adult	97	MDRD, CG, Nankivell, Own	58.1±25.6 (DTPA)	Own (−0.3)	Own (22.4)	Own (42)	
White [41] (2007)	Adult	198	MDRD, CG, Nankivell, Filler, Le Bricon, Rule	59±21 (DTPA)	Filler (0.2)	Le Bricon/Rule (11.2)	Le Bricon (89)	Filler (44)
White [42] (2005)	Adult	117	MDRD, CG, Nankivell, Walser, Jelliffe, Mawer, Filler, Le Bricon, Hoek, Larsson	58±23 (DTPA)	Nankivell (−1.4)	Filler (11.4)	Le Bricon (89)	Le Bricon/Filler (42)
Maillard [43]	Adult	120	MDRD, Filler, Le Bricon, Hoek, Larsson, Rule	52.6±19.4 (inulin)	Le Bricon (2.8)	Hoek/Le Bricon (13.1)	Hoek (82)	
Zahran[b] [44]	Adult	103	MDRD, CG, Bjornsson, Davis, Edwards, Gates, Hull, Jelliffe, Mawer, Nankivell, Salazar, Walser, Le Bricon, Hoek, Filler, Larsson, Grubb, Macias, Rule	46.4±20.7 (inulin)	Gates (−0.2)		Gates (66)	
Poge [45] (2006)	Adult	108[c]	MDRD, Hoek, Filler, Larsson	39.5 (DTPA)	Hoek (−0.6)	Hoek (8.9)	Larsson/Hoek (77)	Hoek (33)
Poge [46] (2008)	Adult	108	Hoek, Own	39.5 (DTPA)	Own (−0.04)	Hoek (8.9)	Own (82)	Own (35)
Filler [6]	Child	536[c]	Schwartz, Own	103±41 (DTPA)	Own (0.3)			
Bouvet [20]	Child	100[e]	Schwartz, Filler, Own	95 (EDTA)	Schwartz	Own	Own (82)	Own (39)
Zappitelli [8]	Child	103[f]	Schwartz, Bokencamp, Filler, Grubb, Own	73.6±35.7 (iothalamate)	Bokencamp (0)		Own (87)	
Bacchetta [26]	Child	252[g]	Schwartz, Hoek, Le Bricon, Larsson, Rule, Filler, Zappitelli, Bouvet	101±32 (inulin)	Larsson (0)	Hoek, Le Bricon, Rule, Zappitelli (19)	Zappitelli, Hoek, Rule (91)	Zappitelli (48)
Huang [47]	Adult	42[h]	MDRD, CKD-EPI, Filler, Grubb, Hoek, Larsson, Le Bricon, Rule	70.9±26.7 (DTPA)	CKD-EPI (−0.6)	Le Bricon (13.2)	Hoek (93)	Hoek (40)
Malignancy								
Blufpand [25]	Child	68	New Schwartz, Filler	113.2±25.3 (inulin)	Filler (7.3)		Filler (82)	
Chew [33]	Adult	57	MDRD, CG, Hoek, Le Bricon, Rule, Larsson, MacIsaac	32–136 (DTPA)	Hoek (−1.0)		Hoek (89)	

MDRD Modification of Diet in Renal Disease equation; CG Cockcroft-Gault equation; CKD-EPI Chronic Kidney Disease Epidemiology Collaboration; Own authors developed their own formula

[a] SD difference, SD of difference between measured and estimated GFR

[b] Indicates used a non-nephelometric cystatin C measurement technique

[c] 11% combined kidney/pancreas transplant and 3 % combined liver/kidney transplant

[d] 5.4 % kidney transplant

[e] 48% kidney transplant

[f] 27% kidney transplant and 7 % other transplant

[g] 17% kidney transplant, 7 % liver transplant, 2 % heart transplant, and 3 % hematopoietic stem cell transplant

[h] 38% liver transplant and 2 % kidney transplant

overestimated GFR (mean bias of 10.2 and 8.0 ml/min/1.73 m^2, respectively).

Equations including cystatin C and creatinine have demonstrated improved accuracy and precision compared to those including only cystatin C [8, 20]. The Bouvet and combined Zappitelli formulas, however, markedly overestimated GFR in our cohort. Systematic biases evident in these formulas may be secondary to variation in creatinine assay methods or endogenous creatinine production between study populations. For example, creatinine-based GFR estimations have overestimated renal function in pediatric liver transplant recipients and children with malignancy [25, 27], possibly due to low serum creatinine from muscle wasting. Additionally, Bouvet et al. used a non-compensated Jaffe method for creatinine measurement, whereas all others (including the present study) used an enzymatic assay. Smaller creatinine values yielded by enzymatic techniques likely contributed to the overestimation of GFR by the Bouvet formula. These limitations of creatinine notwithstanding, the New CKiD formula performed quite well, demonstrating the best 30 and 10 % accuracy among all formulas. Our study is the first, therefore, to validate its use in a cohort composed primarily of children with malignancy or solid organ transplantation.

The strengths of our analysis include the use of an established nuclear medicine technique as the gold standard and measuring cystatin C on the same sample as the NucGFR, thereby reducing intra-patient variability which is often a concern with these measurements [28]. Furthermore, we used an accurate nephelometric assay for the measurement of cystatin C, which has been shown to be more reliable than other techniques, such as those based on turbidimetry [1]. In addition, we used an IDMS-traceable, enzymatic assay to measure serum creatinine, similar to those used by Zappitelli et al. and the 2009 CKiD study. Our patient population had a wide range of GFR (17–147 ml/min/1.73 m^2), whereas prior studies have been limited by including patients with narrower ranges of renal function (Table 6). Finally, we included all patients having a formal GFR measurement at our institution, reducing selection bias. As these tests were ordered for clinical indications, our cohort represented a heterogeneous group of patients at high risk for kidney dysfunction.

Several limitations of our study deserve consideration. First, our cohort was composed of many liver transplant recipients and patients with a history of malignancy. In these subjects, variations in endogenous cystatin C production may have affected the performance of cystatin C-based estimations. For example, elevated cystatin C levels have been demonstrated in patients with active liver disease (cirrhosis, hepatitis) and leukemia [29–31], potentially underestimating GFR when cystatin-C based equations are used. However, cystatin-C based equations have not demonstrated a consistent bias in clinically stable liver transplant recipients and patients with malignancy, although variability in performance has been demonstrated among individual formulas [24, 25, 32–34]. Second, we used ^{99m}Tc-DTPA plasma disappearance curves as our 'gold standard' of GFR measurement, thereby differing from previous studies that measured GFR by iohexol or iothalamate clearance. However, GFR estimation using ^{99m}Tc-DTPA has demonstrated close agreement with iohexol, iothalamate and inulin [15, 35, 36]. Lastly, our last plasma sample was drawn at 210 min post-injection, whereas other studies have used longer intervals of up to 300 min [37]. Shorter sampling intervals can cause an overestimation of GFR in patients with severe renal dysfunction. However, as only one patient in our cohort had a GFR of <30 ml/min/1.73 m^2, this effect was likely to be insignificant.

In summary, our results demonstrate that the Rule, Hoek and Zappitelli cystatin C-based equations and the New CKiD formula provided a good estimation of the GFR in children at high risk of developing renal insufficiency, such as after transplant. While these GFR estimating equations are unlikely to replace gold standard methods in all clinical situations, they offer reasonable accuracy, improved cost and patient convenience compared to formal isotope techniques.

Conflict of Interest statement There are no conflicts of interest to disclose.

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