

# Estimating GFR in Adult Patients with Hematopoietic Cell Transplant: Comparison of Estimating Equations with an Iohexol Reference Standard

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## Abstract

**Background and objectives** Formal evaluation of kidney function before and after hematopoietic cell transplant is important to determine conditioning regimens, type of transplant, and medication dosing. Serum creatinine and estimating equations may not accurately assess kidney function.

**Design, study, participants, & measurements** Existing estimating equations for GFR were compared with an iohexol measure of GFR in a prospective cohort study of 50 patients undergoing hematopoietic cell transplant and subsequent care at the Fred Hutchinson Cancer Research Institute from 2009 to 2013. Patients underwent iohexol GFR, serum creatinine, and cystatin C determination at baseline and day 100 posthematopoietic cell transplant. Iohexol GFR measurements were compared with the CKD Epidemiology Collaboration, Inker CKD Epidemiology Collaboration cystatin C with and without serum creatinine, Modification of Diet in Renal Disease, and Cockcroft–Gault estimating equations using Bland–Altman analysis and McNemar’s test. The iohexol measurements were also compared with blood samples collected simultaneously on filter paper.

**Results** Mean differences between iohexol GFR and eGFR on the basis of Bland–Altman analyses ranged from  $-20.6$  to  $+15.4$  ml/min per  $1.73$  m<sup>2</sup> at baseline and  $-12.7$  to  $+12.9$  ml/min per  $1.73$  m<sup>2</sup> at day 100. The CKD Epidemiology Collaboration and Modification of Diet in Renal Disease estimating equations classified 64% of patients with a GFR < 90 at baseline compared with 38% by iohexol GFR ( $P=0.003$  and  $P<0.01$ , respectively). No statistically significant differences were seen at day 100. The filter paper GFR had a mean difference of 0 at baseline and 5.9 at day 100. Additionally, 21%–37% and 57%–89% of eGFRs were within 10% and 30%, respectively, of the iohexol GFR at baseline, and 16%–34% and 72%–84% were within 10% and 30%, respectively, of the iohexol GFR at day 100; 98% of the filter paper estimates at baseline were within 30%, and 46% were within 10% of iohexol GFR.

**Conclusions** The estimating equations are neither accurate nor precise in the hematopoietic cell transplant population, and clinical decision may require measurement of GFR.

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## Introduction

Decisions regarding the components of hematopoietic cell transplantation (HCT; conditioning regimens, type of transplant [reduced intensity versus myeloablative], and post-HCT immunosuppressive drugs) are often on the basis of kidney function. Traditional methods to measure kidney function and GFR in this patient population are almost exclusively on the basis of serum creatinine (Cr), despite its inaccuracy in patients with mild renal insufficiency, malnutrition, muscle wasting, or cancer and the elderly (1,2). Patients undergoing an HCT may have fluctuations in their nutritional status, muscle mass, and weight that influence serum Cr and derived estimating equations (3,4). Patients are also routinely given prophylactic trimethoprim/sulfamethoxazole post-HCT, which interferes with tubular transport of Cr and may lead to increases in serum Cr that do not reflect actual changes in GFR (5).

One alternative to Cr-based estimates is cystatin C (CysC), a cysteine protease inhibitor expressed by all nucleated cells and freely filtered by the glomerulus. Although serum CysC correlates well with measured GFR and may more accurately measure kidney function than serum Cr in the elderly, patients with cancer, patients with diabetes, and recipients of renal transplants, it is influenced by age, sex, inflammation, calcineurin inhibitors, and prednisone use (2,6–10).

The most accurate measurements of GFR use clearance of substances, such as inulin, EDTA, technetium-99-diethylenetriamine penta-acetic acid, iothalamate, or iohexol. However, these studies are expensive and time intensive, which limits their clinical usefulness. On the basis of the difficulties inherent in using serum Cr and the importance of an accurate measure of kidney function for clinical management of patients undergoing HCT, we compared Cr- and CysC-based GFR-estimating

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equations with GFR measured by plasma iohexol disappearance. The objective of this study was to identify an accurate clinical method for evaluating GFR in patients with HCT, with iohexol GFR (iGFR) as the reference standard. We also compared blood spots placed on filter paper to calculate GFR in patients as a potential alternative method to measured GFR, where patients could collect the 2- and 5-hour time points at home after receiving the iohexol infusion at their physician's office or infusion center. The filter paper could then be mailed in for analysis.

## Materials and Methods

### Patient Selection

We conducted a prospective cohort study of patients undergoing an HCT at the Fred Hutchinson Cancer Research Center from 2009 to 2013. Patients >2 years of age at the time of transplant who were receiving their follow-up care in Seattle were eligible for this study. Patients were excluded if they had an allergy to iodine or were unable to return to Seattle for the 1-year follow-up visit. The study was reviewed and approved by our Institutional Review Board, and all participants and parents provided written informed consent. Only baseline and day 100 data in the adults are presented in this paper.

### Technique of HCT

All patients undergoing HCT received a preparative conditioning regimen followed by infusion of donor hematopoietic cells. Myeloablative regimens were cyclophosphamide-based (with either total body irradiation [TBI] or targeted busulfan) for allogeneic transplants; recipients of autologous grafts received a number of different regimens. Reduced-intensity conditioning regimens consisted of fludarabine and TBI at 2–4 Gy (11). The kidneys are not shielded during TBI. Recipients of allogeneic grafts received prophylaxis against acute graft-versus-host disease (GVHD) with cyclosporin or tacrolimus plus methotrexate, sirolimus, or mycophenolate mofetil (12). These immunosuppressive agents are tapered off around day 80 if there is no evidence of GVHD. Prophylaxis for infections included acyclovir, trimethoprim/sulfamethoxazole, oral fluconazole or itraconazole, and ganciclovir (13–17). Prophylactic ursodiol was given routinely (18).

### Iohexol Determination of GFR

Patients underwent measurement of GFR using plasma clearance of iohexol at baseline (before the conditioning regimen for HCT) around day +100 after HCT (day +30 for recipients of autologous transplants) and again at 1 year after HCT. At each study visit, demographic and clinical variables, including height, weight, vital signs, and prescription medication histories, were recorded. Blood samples were collected before iohexol infusion for hematocrit, serum Cr, iohexol blank, and CysC. GFR was measured by a two-point iohexol plasma disappearance analysis using a one-compartment model approximation validated in both children and adults (19,20). After a time-zero blood sample was obtained, patients received a 5-ml intravenous injection of iohexol from a preweighed syringe (Omnipaque 300 GE Healthcare Amersham Division, Princeton, NJ). The exact start time of the infusion was recorded. The syringe containing

iohexol was weighed to the nearest 0.1 g before and after injection to calculate the dose of iohexol. Blood was drawn at approximately 120 and 300 minutes after the iohexol infusion from a second intravenous catheter placed on the day of the study or the opposite port from the iohexol infusion port from the patient's double-lumen Hickman line. Blood was additionally placed on filter paper (Schleicher and Schuell 903 filter paper) using a syringe ( $n=47$  filter paper at baseline and  $n=34$  at day 100). In three participants, a finger-prick method was used to place blood on filter paper. GFR was computed from the iohexol dose divided by the area under the disappearance curve and scaled for body surface area of  $1.73 \text{ m}^2$  (19,20). All blood samples were collected in serum separator tubes, inverted 5–10 times, and allowed to clot before being centrifuged at 3000 rpm (1187 $\times$ g) for 10 minutes. Serum was stored at  $-80^\circ\text{C}$  until analysis.

Quantification of iohexol concentration in sera and dried blood spots (DBSs) was determined by HPLC in the University of Rochester Medical Center Toxicology Laboratory (21,22). Serum standards were prepared from dilutions of iohexol from the same lot. The interassay coefficients of variation of five separate runs of quality control iohexol samples were 1.1% at a level of 129 mg/L and 2.8% at a level of 12.9 mg/L. The intra-assay coefficients of variation obtained from spiked iohexol samples on three separate studies averaged 1.95% at 14.77 mg/L and 1.23% at 99.25 mg/L. The limit of quantification was 2 mg/L. For the DBS on filter paper, iohexol was quantitatively extracted from the paper according to the method by Niculescu-Duvaz *et al.* (23). A 6.3-mm-diameter punch was taken out of a DBS from the paper to create a disk containing 11.6  $\mu\text{L}$  blood. DBSs were assayed in duplicate, and the average value was reported; approximately four blood spots were obtained at the 120- and 300-minute time points. The disk was placed in a microcentrifuge tube, and iohexol was eluted in 5% perchloric acid during and after sonication. One hundred microliters were injected into the HPLC column. Standards from stock iohexol were prepared at comparable dilutions for calibration. Any sample for which the duplicates differed by >20% was reassayed; if the duplicates differed by >20%, then all samples for that participant were reassayed. If the concentration was <10 mg/L, that time-point sample was re-assayed by doubling the number of disks used. Because iohexol is not taken up by red blood cells, the plasma iohexol concentration was calculated from the whole-blood concentration by correcting for the hematocrit by  $\text{iohexol}/(1 - \text{hematocrit})$  (23). For DBS, the coefficient of variation was 4.3% at a level of 10 mg/L, 3.0% at a concentration of 100 mg/L, and 5.2% at a concentration of 500 mg/L. The average measured/target percentage ranged from 9.6% at 10 mg/L to 91.7% at 100 mg/L to 88.7% at 500 mg/L. The assay was linear over the range of 10–680 mg/L, with a slope of 1.06 and an  $R^2$  of 0.99. Cr was measured using a photometric, kinetic modification of the Jaffe procedure with a Beckman Coulter Cr reagent on a Beckman Coulter AU680 or AU640 analyzer. The methodology is isotope dilution mass spectrometry traceable. CysC was measured at the University of Rochester using a Siemens BN II nephelometer; the assay was performed with a six-point calibration generated from multiple dilutions of a human CysC calibrator obtained from

human urine. The intensity of the signal is proportional to the CysC sample concentration. Each run included one to three sera of known CysC concentration to rule out drift of the assay. Each run of 10–60 samples was preceded and followed by measurements of quality controls of low (1.06 mg/L) and high (1.93 mg/L) CysC concentrations, and the runs were discarded if the quality controls differed from the listed concentrations by >6%. The assay range is 0.195–7.330 mg/L; the reference range for young healthy persons ranges from 0.53 to 0.95 mg/L. The interassay coefficient of variation is 2.3%–3.1% (24).

**Statistical Methods**

We examined the association between five clinically used equations to estimate GFR and GFR as measured by iohexol clearance. The equations used included CKD Epidemiology Collaboration (CKD-EPI), CKD-EPI Cr-CysC, CKD-EPI CysC (25), Cockcroft–Gault CrCl (26), and Modification of Diet in Renal Disease (MDRD) (27).

The bias of each estimating equation was defined as the mean difference between the relevant eGFR and the corresponding iGFR. Bland–Altman plots were used to display this bias along with 95% confidence limits for the mean difference (28). The accuracy of each estimating equation with respect to the corresponding iGFR was

assessed by estimating the proportion of eGFR measurements that were within 10% and 30%, respectively, of the corresponding iGFR measurement. McNemar’s test was used to compare these proportions between each pair of eGFR equations. These analyses were conducted separately among the baseline and the day 100 measurements. We assessed the agreement between iGFR and each eGFR value when categorized as <90 versus ≥90 ml/min per 1.73 m<sup>2</sup> at baseline and day 100. The proportion of abnormal values (defined as <90 ml/min per 1.73 m<sup>2</sup>) by iGFR was compared with that by eGFR using McNemar’s test. Sensitivity and specificity were estimated from these tables.

**Results**

Figure 1 shows patient enrollment. There were no differences noted in demographic data between enrollees and those who refused participation. Demographic and clinical data are presented in Table 1. The majority of our patients did not have significant renal dysfunction at baseline (mean iGFR =99.7 ml/min per 1.73 m<sup>2</sup>). The median age of the cohort was 55 years old, 76% were men, and 92% were Caucasian. The indication for transplant in the majority of patients was a hematologic malignancy. Forty patients (80%) received an allogeneic transplant. Four patients provided baseline study specimens but were not

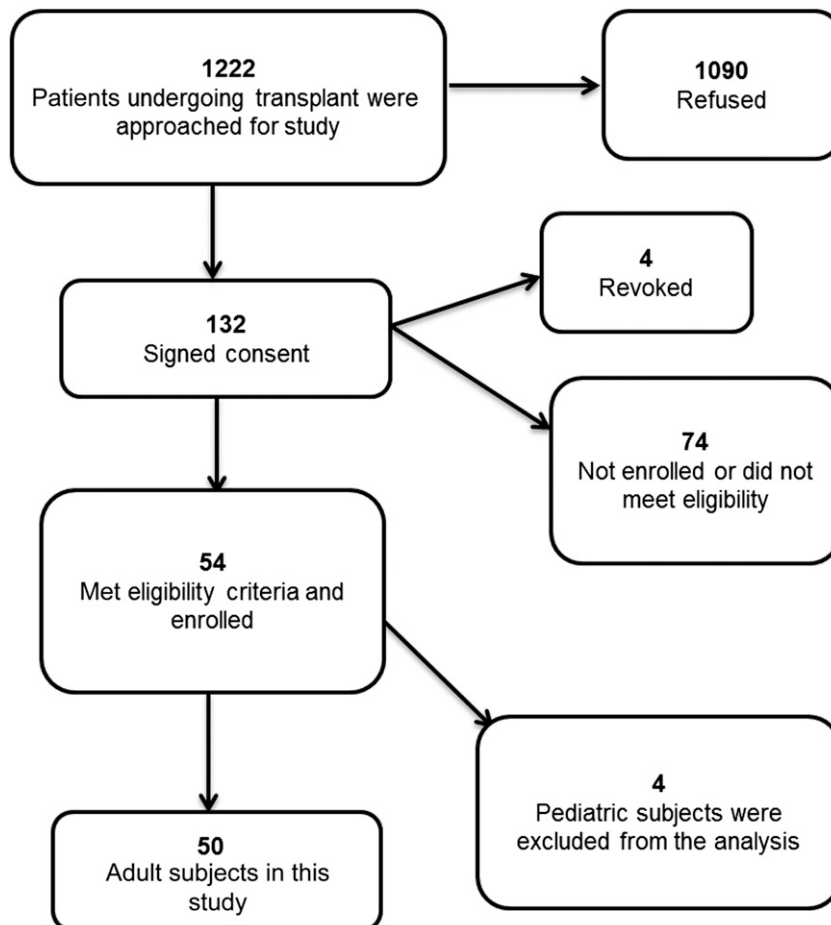


Figure 1. | CONSORT diagram of patient enrollment.

**Table 1. Patient demographic data and clinical characteristics at baseline (before start of conditioning regimen) and around day 100 after hematopoietic cell transplantation**

Characteristic	Baseline (n=50)	Approximately 100 days (n=35)
<b>Age at HCT (yr)</b>		
18–39	9 (18)	6 (17)
40–59	26 (52)	18 (52)
≥60	15 (30)	11 (31)
Median	55	55
Range	23–72.3	23–69
<b>Sex</b>		
Men	38 (76)	28 (80)
Women	12 (24)	7 (20)
<b>Race</b>		
Caucasian	46 (92)	33 (94)
Hispanics	1 (2)	1 (3)
Other	2 (4)	1 (3)
Not available	1 (2)	0 (0)
<b>Diagnosis for HCT</b>		
AML	15 (30)	8 (23)
ALL	6 (12)	5 (14)
CLL	5 (10)	5 (14)
CML	4 (8)	1 (3)
MDS	6 (12)	6 (16)
NHL	6 (12)	3 (9)
MM	2 (4)	2 (6)
Myelofibrosis	3 (6)	2 (6)
Other	3 (6)	3 (9)
<b>Donor type</b>		
Allogeneic (related matched)	11 (22)	11 (31)
Allogeneic (related mismatched)	4 (8)	3 (9)
Autologous	6 (12)	4 (11)
Unrelated donor	24 (48)	17 (49)
Not transplanted	5 (10)	0 (0)
<b>Conditioning regimen</b>		
Reduced intensity regimens	12 (24)	10 (29)
Myeloablative		
CY/TBI 12–13.5 cGy	9 (18)	5 (14)
BU/CY only	12 (24)	7 (20)
Other myeloablative regimens	17 (34)	13 (37)
Mean body mass index (kg/m <sup>2</sup> )	29.6±5.4	27.2±4.3
Mean weight (kg)	88±22.2	82±18.0
Mean BSA	2.02±0.3	1.96±0.3
<b>Hypertensive and on medication</b>		
Yes	5 (10)	24 (69)
<b>On steroid</b>		
Yes	3 (6)	19 (54)
<b>On sulfamethoxazole/trimethoprim</b>		
Yes	22 (44)	22 (63)
<b>Diabetes</b>		
Yes	0 (0)	5 (14)
No	50 (100)	30 (86)

**Table 1. (Continued)**

Characteristic	Baseline (n=50)	Approximately 100 days (n=35)
<b>ACR (mg/g creatinine)</b>		
<30	—	15 (43)
30–299	—	8 (23)
≥300	—	4 (11)
Not available	—	8 (23)
<b>aGVHD grade</b>		
0 or I	—	8 (23)
II	—	18 (51)
III or IV	—	2 (6)
Not graded	—	5 (14)
Autologous	—	2 (6)
<b>cGVHD</b>		
Yes	—	15 (43)
No	—	13 (37)
Not available	—	7 (20)

HCT, hematopoietic cell transplantation; AML, acute myelogenous leukemia; ALL, acute lymphocytic leukemia; CLL, chronic lymphocytic leukemia; CML, chronic myelogenous leukemia; MDS, myelodysplastic syndrome; NHL, non-Hodgkins lymphoma; MM, multiple myeloma; CY, cytoxan; TBI, total body irradiation; BU, busulfan; BSA, body surface area; ACR, albumin-to-creatinine ratio; aGVHD, acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease.

transplanted. Only five patients (10%) of the cohort were hypertensive and on medications at baseline compared with 69% at day 100 post-transplant (Table 1).

Table 2 lists summary values of the iGFR and five commonly used estimating equations to calculate GFR(eGFR) and the estimated bias (and 95% confidence interval for the bias) of each eGFR separately for baseline and day 100 values. The majority of the equations underestimated GFR with the exception of the Cockcroft–Gault equation, which overestimated GFR. This overestimation may be caused by a lack of scaling to body surface area. DBS iGFR was similar to that measured by the venous samples (iGFR) at baseline, with a slight overestimation at day 100. In addition to the bias of each estimating equation, one can see that spread of the individual biases is quite wide, which is evidenced by the relatively large SDs, suggesting a relative lack of precision in the estimating equations, even when the bias is close to zero.

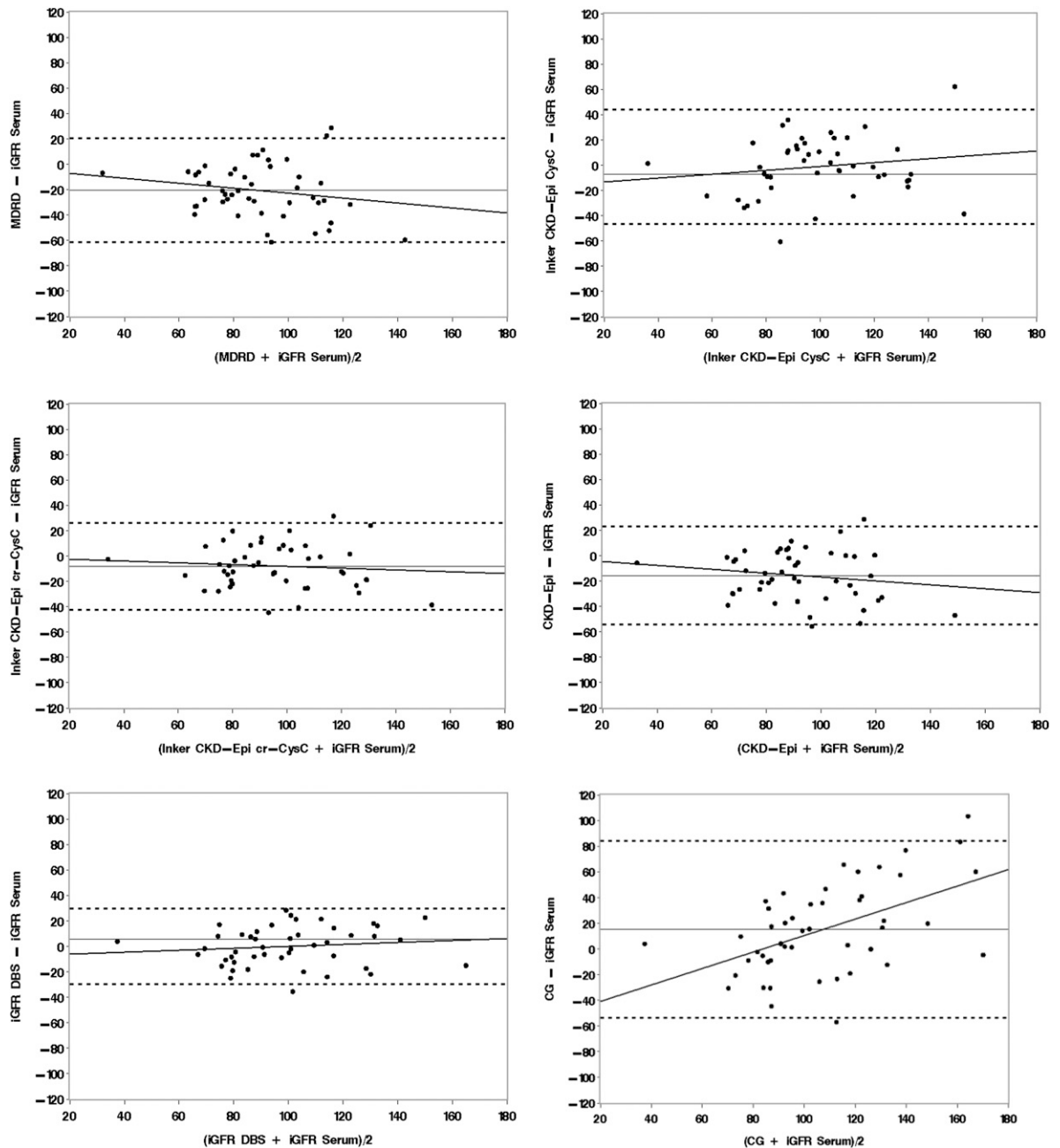
These biases (and the spreads of the individual biases) are further shown in Figures 2 and 3, where Bland–Altman plots are shown for each estimating equation at baseline and day 100. The solid horizontal lines in Figures 2 and 3 are consistent with the values in Table 2, showing a negative bias for each estimating equation other than Cockcroft–Gault and DSB iGFR. When the Cockcroft–Gault equation is adjusted for body surface area, the mean bias is  $-5.1$  ( $-12.6$  to  $2.5$ ) at baseline and  $-1.8$  ( $-9.9$  to  $6.3$ ) at day 100. A least-squares regression line was also fit through the data points in the Bland–Altman plots (Figures 2 and 3). For estimating equations with regression lines that show a negative slope, as the average value of GFR (averaged between iGFR and eGFR) increases, the difference between eGFR and iGFR

**Table 2. Mean value for each measure of GFR as well as the mean difference (±SD and 95% confidence interval) for the difference between each eGFR and the corresponding iohexol GFR**

Measure	Baseline				Day 100			
	Mean (±SD); ml/min per 1.73 m <sup>2</sup>	Mean Difference ±SD (95% CI) between eGFR and iGFR	eGFR within (%) 30% of GFR	eGFR within (%) 10% of GFR	Mean (±SD); ml/min per 1.73 m <sup>2</sup>	Mean Difference ±SD (95% CI) between eGFR and iGFR	eGFR within (%) 30% of GFR	eGFR within (%) 10% of GFR
iGFR	99.9 (±24.6)				86.1 (±28.9)			
CKD-EPI	84.1 (±21.4)	-15.6±19.7 (-21.4 to -9.8)	79	34	77.4 (±24.1)	-8.0±20.0 (-15.1 to -0.9)	82	33
Inker CKD-EPI	91.9 (±23.2)	-7.9±17.5 (-13.1 to -2.7)	89	33	76.4 (±27.1)	-8.2±19.2 (-15.1 to -1.3)	84	16
Cr-CysC								
Inker CKD-EPI	98.7 (±28.0)	-1.1±23.2 (-7.9 to 5.8)	76	37	77.6 (±31.6)	-7.1±23.1 (-15.4 to 1.2)	72	28
CysC								
Cockcroft-Gault <sup>a</sup>	115.2 (±40.1)	15.4±35.2 (5.1 to 25.8)	57	21	98.1 (±36.8)	12.9±30.1 (2.4 to 23.4)	79	32
MDRD	79.2 (±20.8)	-20.6±20.9 (-26.7 to -14.4)	70	23	72.7 (±22.9)	-12.7±20.3 (-19.9 to -5.5)	76	30
iGFR DBS	100.0 (±26.4)	0.0±15.2 (-4.5 to 4.5)	98	46	92.0 (±35.7)	5.9±13.9 (0.8 to 10.9)	97	34

Percentages of eGFR measurements that are within 30% and 10% of the corresponding iGFR are also presented. Results are presented separately for baseline and day 100 pairs. Percentage of eGFR within iGFR was statistically significant ( $P<0.05$ ) only at baseline for Inker CKD-EPI Cr-CysC versus Inker CKD-EPI CysC ( $P=0.03$ ), Inker CKD-EPI Cr-CysC versus Cockcroft-Gault ( $P=0.003$ ), and Inker CKD-EPI Cr-CysC versus MDRD ( $P=0.02$ ); no statistical comparisons were made with iGFR DBS. iGFR, iohexol GFR; CKD-EPI, CKD Epidemiology Collaboration; Cr, creatinine; CysC, cystatin C; MDRD, Modification of Diet in Renal Disease; DBS, dried blood spot; 95% CI, 95% confidence interval.

<sup>a</sup>Units are milliliters per minute.

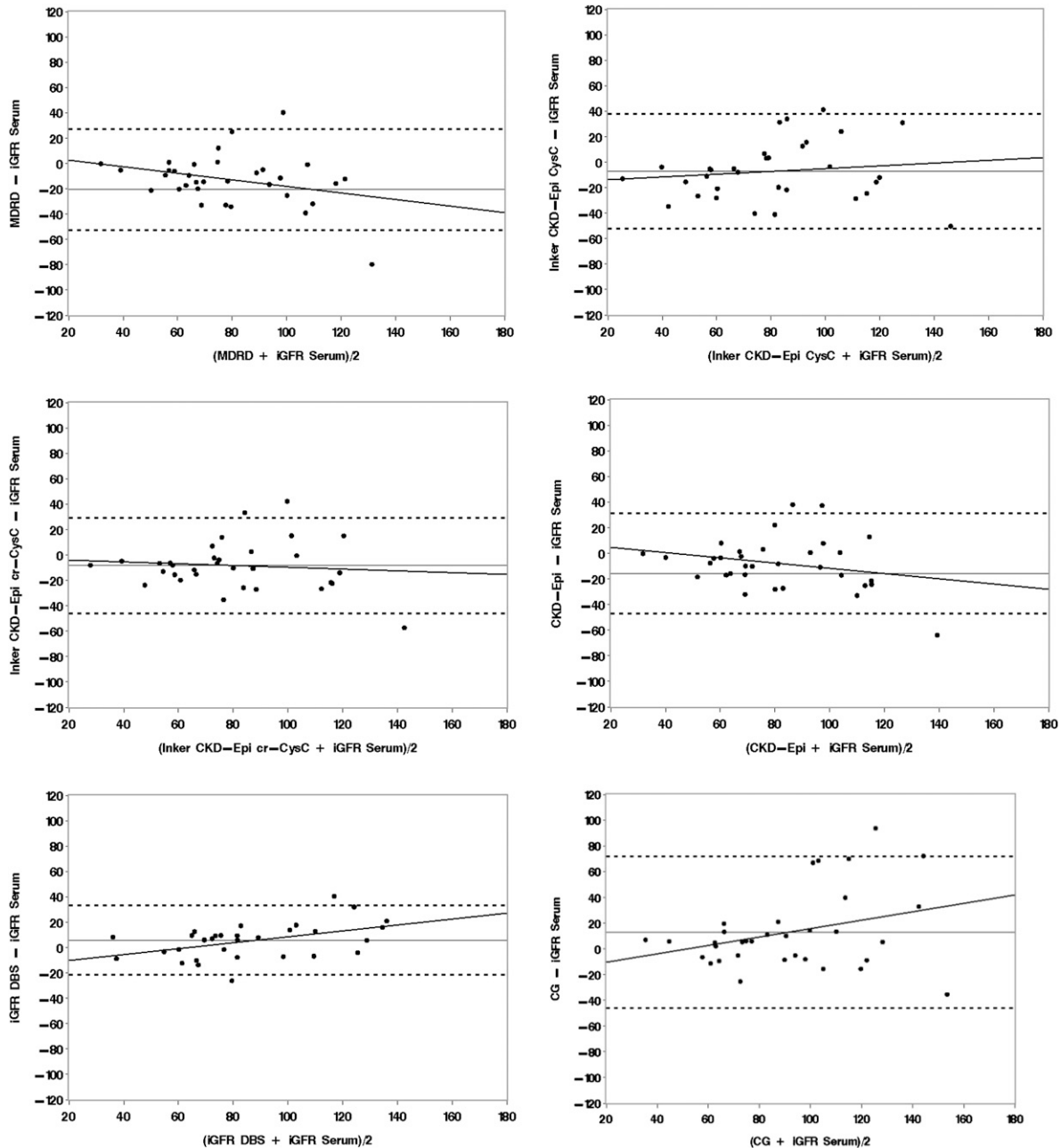


**Figure 2. | Baseline.** Bland–Altman plots for each eGFR relative to corresponding iGFR. Solid horizontal lines represent the mean difference between eGFR and iGFR (bias), and dashed horizontal lines are 95% confidence limits for mean difference. Least squares regression lines are also included. CG, Cockcroft–Gault; CKD-EPI, CKD Epidemiology Collaboration; Cr, creatinine; CysC, cystatin C; DBS, dried blood spot; iGFR, iohexol GFR; MDRD, Modification of Diet in Renal Disease.

becomes more negative. This coupled with a negative mean bias implies that the underestimation of eGFR is larger when the GFR is higher. For estimating equations that have a positive slope to the regression line (Cockcroft–Gault and Inker CKD-EPI CysC), the bias is less negative and/or more positive with a larger average GFR.

The accuracy of each eGFR relative to the corresponding iGFR was assessed by categorizing each eGFR as being within 10% or 30% of the corresponding iGFR. For each estimating equation, <50% (and as low as 16%) of the

eGFR values were within 10% of their corresponding iGFR value at baseline and day 100, suggesting that there is a lack of concordance between these estimating equations and iGFR (Table 2). The level of accuracy when defined as within 30% was obviously higher, ranging from 70% to 89%. Concordance at baseline was generally similar to that at day 100 at the 30% threshold. The concordance of the DBS GFR was nearly perfect when defined as within 30% but not markedly superior to the estimating equations when defined as within 10% of the iGFR.



**Figure 3. | Day 100.** Bland–Altman plots for each eGFR relative to corresponding iGFR. Solid horizontal lines represent the mean difference between eGFR and iGFR (bias), and dashed horizontal lines are 95% confidence limits for mean difference. Least squares regression lines are also included.

In separate analyses, the accuracy of each estimating equation was assessed and summarized by comparing the agreement between iGFR and each eGFR in terms of correctly classifying patients as above or below 90 ml/min per 1.73 m<sup>2</sup>. We compared the proportion of abnormal values <90 by iGFR with the proportion of values <90 by eGFR (Table 3). In some instances, the eGFR classified a higher proportion of patients as <90 ml/min per 1.73 m<sup>2</sup> relative to iGFR, although the only equations for which this was statistically significantly different were CKD-EPI at baseline (63.8% were <90 ml/min per 1.73 m<sup>2</sup> by CKD-EPI versus 38.3% by iGFR; *P*=0.003) and MDRD at baseline

(63.8% by MDRD versus 38.3% by iGFR; *P*<0.01). The DBS GFR measurement led to similar proportions of measurements <90 ml/min per 1.73 m<sup>2</sup> relative to iGFR at baseline and day 100. The adjusted Cockcroft–Gault equation also estimated a higher proportion of patients with a GFR<90 ml/min per 1.73 m<sup>2</sup> at both baseline (48.9%) and day 100 (64.7%) than the iGFR, but this difference was not statistically significant. C-reactive protein was measured in 70% of our population and there was no difference between the eGFR and iGFR for the 2 equations incorporating CysC based on whether C-reactive protein was <3 or ≥3 mg/L (data in Supplemental Material).

Table 3. Concordance of iGFR and eGFR based on the threshold of 90 mL/min/1.73m<sup>2</sup>.

	Proportion with GFR <90 mL/min/1.73m <sup>2</sup>				Sensitivity				Specificity			
	Baseline (%)		P value		Day 100 (%)		P value		Baseline (%)		Day 100 (%)	
	Baseline (%)	P value	Day 100 (%)	P value	Baseline (%)	Day 100 (%)	Baseline (%)	Day 100 (%)	Baseline (%)	Day 100 (%)		
iGFR <sup>a</sup>	38.3	0.003	60.6		16/18 (89)	17/20 (85)	15/19 (52)	11/13 (85)				
CKD-EPI	63.8		57.6									
iGFR	39.1	0.25	62.5	0.41	14/18 (78)	18/20 (90)	20/28 (71)	8/12 (67)				
Inker CKD-EPI + CysC-SCr	47.8		68.6									
iGFR	39.1	0.25	62.5	0.74	10/18 (56)	15/20 (75)	24/28 (86)	8/12 (67)				
Inker CKD-EPI CysC	30.4		59.4									
iGFR	38.3	0.23	61.8	0.06	7/18 (39)	15/21 (71)	23/29 (79)	12/13 (92)				
Cockcroft-Gault	27.7		47.1									
iGFR	38.3	0.005	60.6	0.10	15/18 (83)	18/20 (90)	14/29 (48)	6/13 (46)				
MDRD	63.8		75.8									
iGFR	39.1	0.76	59.4	0.56	12/18 (67)	17/19 (89)	23/28 (82)	12/13 (92)				
iGFR DBS	37.0		56.3									

Proportion of patients with a <90 mL/min/1.73m<sup>2</sup> listed for iGFR and eGFR, as well as sensitivity and specificity for each eGFR with iGFR taken as the gold standard. Listed P value from McNemar's test comparing proportion of tests <90 mL/min/1.73m<sup>2</sup> at baseline and day 100.

<sup>a</sup>The percentage varies as three patients did not have a serum iothexol at baseline and + a cystatin C was not obtainable on two patients.

## Discussion

This prospective study compared five common GFR-estimating equations with an iothexol reference measure of GFR in the HCT population. The majority of our patients did not have significant renal dysfunction at baseline (mean iGFR =99.7 mL/min per 1.73 m<sup>2</sup>). None of the estimating equations provided a sufficiently accurate assessment of kidney function defined as a high percentage of eGFR values that are within 10% of the corresponding iGFR value. Moreover, the SD of the individual biases is relatively large, suggesting relatively imprecise measurements in eGFR. The Inker CKD-EPI CysC equation has a bias that is near zero at baseline, and at day 100, the 95% confidence interval includes zero. However, only 37% and 22% of measurements were within 10% of the iGFR at baseline and day 100, respectively. DBS iGFR provided accurate measurements of GFR with the least bias and the highest proportion of pairs within 10% (46% and 34%) of the corresponding iGFR. It is unclear why there was not better agreement between iGFR and the filter paper method. Differences might be because of errors in applying blood to the filter paper, inaccurate measurement of hematocrit, or variability in calibration.

Studies comparing serum CysC with eGFR measurements in patients with cancer have reported conflicting results but generally found CysC to perform better than serum Cr (7,8,29–31). One adult HCT study evaluated CysC as a measure of renal function (31), finding that elevations in CysC did not correlate with serum Cr or Cr clearance. However, Demirtaş *et al.* (31) did not look at area under the curve, receiver-operator characteristic curves, or 1/CysC curves, which have been shown to be more accurate and correlate better with other measures of GFR (32). In a pediatric HCT study, Cr clearance estimates on the basis of 24-hour urine collections and CysC-based estimating equations were compared with <sup>99m</sup>Tc-DTPA-measured GFR as the reference standard. Hazar *et al.* (33) found no correlation between the estimating equations and their radiolabeled GFR measurement. Other studies in the HCT population have assessed whether estimating equations correlate with 24-hour urine collection for Cr clearance (34).

As with Cr, a major effort is now being made to standardize CysC measurements (35). With regard to our method for measuring CysC (Siemens/Dade Behring immunonephelometric method), Inker *et al.* (36) have recommended increasing Siemens values by approximately 12%, expressing the CKD-EPI CysC equations for estimating GFR with standardized serum CysC values (36). However, Siemens, in a private correspondence to users of the BNII, has recommended increasing all values by 17% (not on the basis of published data). When such corrections are applied to estimates of GFR on the basis of CysC, the estimates are lower and the underestimations are larger than when uncorrected CysC values are applied to the Inker equations. Therefore, we chose not to adjust the CysC measurements by the 12% or 17% suggested, because there is no agreement on the specific size of the correction for Siemens immunonephelometry.

The limitations of this study include the small sample size and the loss to follow-up of patients over time. In addition, patients are often on prednisone, which can affect CysC levels.

Determining the most accurate measure of kidney function is imperative in this patient population. Decisions



regarding dosing of the components of conditioning regimens and type of transplant as well as medication dosing post-HCT, especially of calcineurin inhibitors, are often on the basis of renal function. Additional medications commonly dose-adjusted on the basis of GFR include cyclophosphamide, fludarabine, ganciclovir, and levofloxacin. Reduced intensity conditioning regimens that use high-dose fludarabine require an accurate GFR. In addition, certain protocols, specifically cord blood transplants and transplants for nonmalignant causes, exclude patients with an eGFR <60, because high doses of calcineurin inhibitors are used as prophylaxis against GVHD in the first 30 days post-transplant. Without an accurate assessment of renal function, patients are at risk for under- or overdosing of medications and may be denied certain treatment protocols if the GFR is estimated to be <60 ml/min per 1.73 m<sup>2</sup>. The filter paper methodology warrants additional study, because it offers a relatively simple finger-prick method that could be done at home by patients after receiving the iothexol infusion at a medical facility. It would eliminate the long iothalamate studies and 24-hour urine collections currently used. Given the bias and inaccuracy of the estimating equations, we recommend that, when eligibility criteria for certain treatment protocols are on the basis of a GFR threshold of patients, a standard measure of GFR, such as iGFR, be performed.

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#### Disclosures

None.

#### References

- Beddhu S, Samore MH, Roberts MS, Stoddard GJ, Pappas LM, Cheung AK: Creatinine production, nutrition, and glomerular filtration rate estimation. *J Am Soc Nephrol* 14: 1000–1005, 2003
- Ross EA, Wilkinson A, Hawkins RA, Danovitch GM: The plasma creatinine concentration is not an accurate reflection of the glomerular filtration rate in stable renal transplant patients receiving cyclosporine. *Am J Kidney Dis* 10: 113–117, 1987
- Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D; Modification of Diet in Renal Disease Study Group: A more accurate method to estimate glomerular filtration rate from serum creatinine: A new prediction equation. *Ann Intern Med* 130: 461–470, 1999
- Fliser D, Franek E, Joest M, Block S, Mutschler E, Ritz E: Renal function in the elderly: Impact of hypertension and cardiac function. *Kidney Int* 51: 1196–1204, 1997
- Delanaye P, Mariat C, Cavalier E, Maillard N, Krzesinski JM, White CA: Trimethoprim, creatinine and creatinine-based equations. *Nephron Clin Pract* 119: c187–c193, discussion c193–c194, 2011
- Dharnidharka VR, Kwon C, Stevens G: Serum cystatin C is superior to serum creatinine as a marker of kidney function: A meta-analysis. *Am J Kidney Dis* 40: 221–226, 2002
- Al-Tonbary YA, Hammad AM, Zaghloul HM, El-Sayed HE, Abu-Hashem E: Pretreatment cystatin C in children with malignancy: Can it predict chemotherapy-induced glomerular filtration rate reduction during the induction phase? *J Pediatr Hematol Oncol* 26: 336–341, 2004
- Séronie-Vivien S, Toullec S, Malard L, Thomas F, Durrand V, Chatelut E: Contribution of the MDRD equation and of cystatin C for renal function estimates in cancer patients. *Med Oncol* 23: 63–73, 2006
- Visvardis G, Griveas I, Zilidou R, Papadopoulou D, Mitsopoulos E, Kyriklidou P, Manou E, Ginikopoulou E, Meimaridou D, Pavlitou A, Sakellariou G: Glomerular filtration rate estimation in renal transplant patients based on serum cystatin-C levels: Comparison with other markers of glomerular filtration rate. *Transplant Proc* 36: 1757–1759, 2004
- Stevens LA, Schmid CH, Greene T, Li L, Beck GJ, Joffe MM, Froissart M, Kusek JW, Zhang YL, Coresh J, Levey AS: Factors other than glomerular filtration rate affect serum cystatin C levels. *Kidney Int* 75: 652–660, 2009
- Carella AM, Champlin R, Slavin S, McSweeney P, Storb R: Mini-allografts: Ongoing trials in humans. *Bone Marrow Transplant* 25: 345–350, 2000
- Doroshov JH, Synold TW: Pharmacologic basis for high-dose chemotherapy. In: *Thomas' Hematopoietic Cell Transplantation*, edited by Appelbaum F, Forman S, Negrin R, Blume K, West Sussex, United Kingdom, Chichester, 2009, pp 287–315
- Leather HL, Wingard JR: Bacterial infections. In: *Thomas' Hematopoietic Cell Transplantation*, Oxford, United Kingdom, Wiley-Blackwell, 2009, pp 1325–1345
- Zaia JA: Cytomegalovirus infection. In: *Thomas' Hematopoietic Cell Transplantation*, Oxford, United Kingdom, Wiley-Blackwell, 2009, pp 1367–1381
- Brown JMY: Fungal infections after hematopoietic cell transplantation. In: *Thomas' Hematopoietic Cell Transplantation*, Oxford, United Kingdom, Wiley-Blackwell, 2009, pp 1346–1366
- Ito JI: Herpes simplex virus infections. In: *Thomas' Hematopoietic Cell Transplantation*, Oxford, United Kingdom, Wiley-Blackwell, 2009, pp 1382–1387
- Ho DY, Arvin AM: Varicella-zoster virus infections. In: *Thomas' Hematopoietic Cell Transplantation*, Oxford, United Kingdom, Wiley-Blackwell, 2009, pp 1388–1409
- Ruutu T, Eriksson B, Remes K, Juvonen E, Volin L, Remberger M, Parkkali T, Hägglund H, Ringdén O; Nordic Bone Marrow Transplantation Group: Ursodeoxycholic acid for the prevention of hepatic complications in allogeneic stem cell transplantation. *Blood* 100: 1977–1983, 2002
- Ng DK, Schwartz GJ, Jacobson LP, Palella FJ, Margolick JB, Warady BA, Furth SL, Muñoz A: Universal GFR determination based on two time points during plasma iothexol disappearance. *Kidney Int* 80: 423–430, 2011
- Berg UB, Bäck R, Celsi G, Halling SE, Homberg I, Krmar RT, Monemi KA, Oborn H, Herthelius M: Comparison of plasma clearance of iothexol and urinary clearance of inulin for measurement of GFR in children. *Am J Kidney Dis* 57: 55–61, 2011
- Schwartz GJ, Furth S, Cole SR, Warady B, Muñoz A: Glomerular filtration rate via plasma iothexol disappearance: Pilot study for chronic kidney disease in children. *Kidney Int* 69: 2070–2077, 2006
- Schwartz GJ, Muñoz A, Schneider MF, Mak RH, Kaskel F, Warady BA, Furth SL: New equations to estimate GFR in children with CKD. *J Am Soc Nephrol* 20: 629–637, 2009
- Niculescu-Duvaz I, D'Mello L, Maan Z, Barron JL, Newman DJ, Dockrell ME, Kwan JT: Development of an outpatient finger-prick glomerular filtration rate procedure suitable for epidemiological studies. *Kidney Int* 69: 1272–1275, 2006
- Schwartz GJ, Schneider MF, Maier PS, Moxey-Mims M, Dharnidharka VR, Warady BA, Furth SL, Muñoz A: Improved equations estimating GFR in children with chronic kidney disease using an immunonephelometric determination of cystatin C. *Kidney Int* 82: 445–453, 2012
- Inker LA, Schmid CH, Tighiouart H, Eckfeldt JH, Feldman HI, Greene T, Kusek JW, Manzi J, Van Lente F, Zhang YL, Coresh J, Levey AS; CKD-EPI Investigators: Estimating glomerular filtration rate from serum creatinine and cystatin C. *N Engl J Med* 367: 20–29, 2012
- Cockcroft DW, Gault MH: Prediction of creatinine clearance from serum creatinine. *Nephron* 16: 31–41, 1976
- Levey AS, Coresh J, Greene T, Stevens LA, Zhang YL, Hendriksen S, Kusek JW, Van Lente F; Chronic Kidney Disease Epidemiology Collaboration: Using standardized serum creatinine values in the modification of diet in renal disease study equation for estimating glomerular filtration rate. *Ann Intern Med* 145: 247–254, 2006

28. Bland JM, Altman DG: Comparing methods of measurement: Why plotting difference against standard method is misleading. *Lancet* 346: 1085–1087, 1995
29. Holweger K, Bokemeyer C, Lipp HP: Accurate measurement of individual glomerular filtration rate in cancer patients: An ongoing challenge. *J Cancer Res Clin Oncol* 131: 559–567, 2005
30. Kleber M, Cybulla M, Bauchmüller K, Ihorst G, Koch B, Engelhardt M: Monitoring of renal function in cancer patients: An ongoing challenge for clinical practice. *Ann Oncol* 18: 950–958, 2007
31. Demirtaş S, Akan O, Can M, Elmali E, Akan H: Cystatin C can be affected by nonrenal factors: A preliminary study on leukemia. *Clin Biochem* 39: 115–118, 2006
32. Perkins BA, Nelson RG, Ostrander BE, Blouch KL, Krolewski AS, Myers BD, Warram JH: Detection of renal function decline in patients with diabetes and normal or elevated GFR by serial measurements of serum cystatin C concentration: Results of a 4-year follow-up study. *J Am Soc Nephrol* 16: 1404–1412, 2005
33. Hazar V, Gungor O, Guven AG, Aydin F, Akbas H, Gungor F, Tezcan G, Akman S, Yesilipek A: Renal function after hematopoietic stem cell transplantation in children. *Pediatr Blood Cancer* 53: 197–202, 2009
34. Hahn T, Yao S, Dunford LM, Thomas J, Lohr J, Arora P, Battiwalla M, Smiley SL, McCarthy PL Jr.: A comparison of measured creatinine clearance versus calculated glomerular filtration rate for assessment of renal function before autologous and allogeneic BMT. *Biol Blood Marrow Transplant* 15: 574–579, 2009
35. Nilsson-Ehle P, Grubb A: New markers for the determination of GFR: Iohexol clearance and cystatin C serum concentration. *Kidney Int Suppl* 47: S17–S19, 1994
36. Inker LA, Eckfeldt J, Levey AS, Leiendecker-Foster C, Rynders G, Manzi J, Waheed S, Coresh J: Expressing the CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) cystatin C equations for estimating GFR with standardized serum cystatin C values. *Am J Kidney Dis* 58: 682–684, 2011

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