Title: Assessment of kidney function using inulin-based and estimated glomerular filtration rates before and after allogeneic hematopoietic stem cell transplantation in pediatric patients

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allo-HSCT	allogeneic stem cell transplantation
GFR	glomerular filtration rate
iGFR	inulin-based GFR
eGFR	estimated GFR
Cr	creatinine
CysC	cystatin C
β2MG	beta-2 microglobulin
24hCcr	24-hour creatinine clearance
BUN	blood urea nitrogen
CKD	chronic kidney disease
aGVHD	acute graft versus host disease
CKiD	Chronic Kidney Disease in Children
LOA	limits of agreement
P10	within 10%
P30	within 30%
TBI	total body irradiation

Abstract

Background: Accurate evaluation of kidney function before and after allogeneic stem cell transplantation (allo-HSCT) is important for both informed decision making and detection of chronic kidney disease. However, no report has evaluated the glomerular filtration rate (GFR) in pediatric patients who underwent HSCT using the gold standard GFR measurement, as well as inulin-based GFR (iGFR).

Methods: We assessed iGFR before and after allo-HSCT to evaluate the impact of allo-HSCT on GFR in a prospective cohort study of 17 pediatric patients. We also assessed the accuracy and bias of the values of estimated GFR (eGFR) calculated using serum creatinine (Cr)-, cystatin C (CysC)-, beta-2 microglobulin (β_2MG)-, 24-hour creatinine clearance (24hCcr)-, and full Chronic Kidney Disease in Children (CKiD) that combines Cr, CysC, and blood urea nitrogen-based equations with iGFR as a reference to identify the most reliable equation for GFR.

Results: There was no significant difference between the values before and after allo-HSCT. CKiD CysC-, 24hCcr-, and full CKiD-based values showed good P30 accuracy (80.6%, 79.3%, and 80.6%, respectively), but only 24hCcr and full CKiD had good mean bias (8.5% and 8.9%, respectively) and narrow 95% limits of agreement (-32.2 to 52.7 mL/min/1.73 m^2 and -29.3 to 47.4 mL/min/1.73 m^2, respectively) compared with the corresponding iGFR.

Conclusion: There was no significant impact of allo-HSCT on GFR in our cohort. The most reliable equations for pediatric patients with allo-HSCT were eGFR-24hCcr and eGFR-full CKiD.

Introduction

Kidney function is one of the most important factors in decision making for allogeneic hematopoietic stem cell transplantation (allo-HSCT) with regards to conditioning regimens, type of transplant, and selection of immunosuppressive drugs and antibiotics. The prevalence of chronic kidney disease (CKD) after allo-HSCT has been reported to be 16.6%.[1] Therefore, accurate assessment of kidney function is essential following allo-HSCT. However, there are no established guidelines for how to assess kidney function before and after allo-HSCT.

Glomerular filtration rate (GFR) is widely accepted as the best overall index of kidney function.[2] Ideally, GFR should be measured with a marker that is freely filtered through the glomerulus and neither secreted nor reabsorbed in the renal tubules. Inulin meets these criteria and its urinary clearance has therefore been regarded as the gold standard for measuring GFR.[2,3] Standard inulin clearance (the inulin-based GFR [iGFR]) is assessed by loading and continuous infusion of inulin with regular collection of urine samples.[4-6] However, no report has evaluated GFR in pediatric patients who underwent HSCT using standard iGFR because this approach has some problems: it is expensive, cumbersome, difficult to dissolve and maintain in a solution, and there is a limited supply.[2] As a result, other methods of measuring GFR, which use bolus injection of markers such as ^{51}Cr-ethylenediaminetetraacetic acid, ^{99}Tc-diethylenetriaminepentaacetic acid, and iohexol have been used to evaluate GFR in HSCT.[7-13]

In clinical practice, estimated GFR (eGFR) which is used by serum levels of endogenous markers is more widely and frequently used to evaluate renal function because measurement of formal GFR is invasive, cumbersome, and an expensive technique. Traditional methods used to measure kidney function and the GFR rely almost exclusively on evaluation of serum creatinine (Cr) to calculate the estimated GFR (eGFR)-Cr, which has been reported to be inaccurate for patients with mild renal insufficiency, malnutrition, and muscle wastage, children, and elderly patients.[14-17] New variations of eGFR, including cystatin C (CysC)- and

beta-2 microglobulin (β_2MG)-based eGFRs, have also been reported to be easy-to-measure markers of renal function,[18-22] although their utility remains unclear, especially in the case of pediatric patients undergoing allo-HSCT.

As measurement of iGFR has been covered by the public medical insurance in Japan since 2006, we can assess iGFR easily. The primary objective of this study was to assess the GFR using renal clearance of inulin before and after allo-HSCT to evaluate the impact of allo-HSCT on GFR in our cohort. We also identified the most reliable method of eGFR for evaluating GFR in pediatric patients with allo-HSCT, with iGFR as the reference standard.

Methods

Study design and settings

We prospectively recruited patients younger than 18 years of age who underwent allo-HSCT from August 2016 to December 2019 in the Pediatric Department of Shinshu University Hospital, which is the only tertiary hospital where allo-HSCT can be performed in the Nagano prefecture of Japan. We explained this study to all patients and their guardians who planned to undergo allo-HSCT. Next, we screened the patients who wanted to participate in our study and excluded those who were unable to control their urination. Basically, the iGFR measurements were performed before and after allo-HSCT (within 20 days before allo-HSCT and within 20 days before discharge from the hospital after allo-HSCT) if the participant provided consent for the measurement. Participants' information was collected until April 2020 for all participants. After discharge from the hospital, all participants who underwent allo-HSCT were followed up in the Pediatric Department of Shinshu University Hospital once every 1 or 2 months. This study and all its protocols were approved by the ethical committee of the Shinshu University School of Medicine (approval number: 3805). Written informed consent was obtained from each patient and/or guardian. The study was carried out in compliance with the

Helsinki Declaration.

Data collection

Clinical data were obtained from the medical record and included age, gender, primary diagnosis, height, weight, conditioning, stem cell source, grades of acute graft versus host disease (aGVHD), use of corticosteroid, amphotericin B, and calcineurin inhibitor, and laboratory data.

Measurements of serum and urine inulin levels and renal clearance

We calculated GFR from the inulin concentration as previously described[23,24] and adjusted it to the body surface area standardized to 1.73 m^2. The inulin clearance was measured using the continuous infusion method from samples taken once per hour, two times in total, with the participant under fasting and hydrated conditions as previously reported.[19,20,25,26] Specifically, the participants fasted overnight and were allowed only water after waking in the morning. All received the Ringer's solution (20 mL/kg body weight, maximum: 500 mL) intravenously for 30 minutes to achieve good diuresis, followed by 5 mL/kg/h until completion of testing (for 150 minutes). Beginning 30 minutes after water loading, inulin was administered intravenously at a priming dose of 40 mg/kg body weight for 30 minutes to achieve an extracellular fluid level of 20 mg/dL. Subsequently, inulin was administered at an appropriate rate to maintain a constant blood inulin concentration. The rate of inulin infusion, therefore, had to equal the rate of inulin loss in the urine, which was calculated using the GFR estimated from the serum Cr concentration by the original Schwartz formula.[27-30] After calculating the body surface area by the Haycock method [31], an inulin load of 0.7 × eGFR mL/m^2/h was administered. Urine samples were collected once per hour, two times in total (60 minutes and 120 minutes after the start of the measurement), during which blood samples were collected twice from an indwelling

cannula (30 minutes and 90 minutes after the start of the measurement). Serum and urine inulin levels were measured by BML Inc. (Tokyo, Japan). Urinary and serum levels of inulin were determined using an enzymatic method on an automated analyzer (JCA-BM9130; JEOL Ltd., Tokyo, Japan) with Dia-color-inulin (Toyobo Co., Ltd., Tokyo, Japan).

Estimation of the glomerular filtration rate

Serum Cr values were evaluated through an enzymatic assay (Shino-Test Co., Kanagawa, Japan) using a BioMajesty™ automated analyzer (JCA-BM6070; JEOL Ltd., Tokyo, Japan) just before the iGFR measurement. The eGFR-Cr was calculated using the original Schwartz formula,[30] new bedside Chronic Kidney Disease in Children (CKiD) formula,[32] and the formula reported by Uemura et al. for Japanese children.[26] For the original Schwartz formula, we used the value of serum Cr defined by the Jaffe method.[30] When using the bedside CKiD or Uemura's formula, we used the value of serum Cr obtained from the enzymatic analysis described earlier. In addition, 24-hour creatinine clearance (24hCcr) was calculated from plasma samples and 24-hour urinary Cr within 3 days of the iGFR measurement. The eGFR-24hCcr was calculated as 24hCcr multiplied by 0.76.[33] Serum CysC values were evaluated through a particle-enhanced immunonephelometric analysis (Nittobo Medical Co., Ltd., Fukushima, Japan) using a BioMajesty™ automated analyzer (JCA-BM6050; JEOL Ltd., Tokyo, Japan) just before the iGFR measurement. We calculated eGFR-CysC using the CKiD cystatin C-only formula[34] and Uemura's formula.[19] Serum β_2MG values were evaluated through a latex agglutination immunoassay (Denka Seiken Co., Ltd., Tokyo, Japan) using a BioMajesty™ automated analyzer (JCA-BM6050; JEOL Ltd., Tokyo, Japan) just before the iGFR measurement. The eGFR-β_2MG was estimated with the formula reported by Ikezumi et al.[20] Blood urea nitrogen (BUN) values were evaluated through a urease-GLDH method (Shino-Test Co., Kanagawa, Japan) using a BioMajesty™ automated analyzer (JCA-BM6070;

JEOL Ltd., Tokyo, Japan) just before the iGFR measurement. The eGFR-full CKiD was estimated with the formula reported by Schwartz et al which includes Cr, CysC, and BUN.[34] The formulae used for calculating GFRs are provided in Table 1.

Table 1: Equations used to estimate the glomerular filtration rate

Creatinine-based formulas*	
Original Schwartz	k × height (cm)/S-Cr, where k is 0.7 for male individuals aged 13-18 years, 0.45 for children aged <1 year, and 0.55 otherwise.
Bedside CKiD	0.413 × height (cm)/S-Cr (mg/dL)
Uemura's formula	110.2 × reference S-Cr (mg/dL)/patient's S-Cr (mg/dL)+2.93
Cystatin C-based formulas	
CKiDcys	$70.69 \times (\text{S-CysC [mg/L]})^{-0.931}$
Uemura's formula	104.1/S-CysC (mg/L) - 7.80
Beta-2 microglobulin-based formula	
Ikezumi's formula	149.0/S-β_2MG (mg/L) + 9.15
24-hour creatinine clearance-based formula	24hCcr × 0.76
24-hour creatinine clearance	U-Cr (mg/dL) × urine volume (mL/day)/S-Cr/1440 × 1.73/BSA
Creatinine- and cystatin C-based formula (full CKiD)	$39.8 \times (\text{height[m]/S-Cr [mg/dL]})^{0.456} \times (1.8/\text{S-CysC [mg/dL]})^{0.418} \times (30/\text{S-BUN [mg/dL]})^{0.079} \times (1.076^{\text{male}})\ (1.00^{\text{female}}) \times (\text{height [m]}/1.4)^{0.179}$

S, serum; Cr, creatinine; CysC, cystatin C; β_2MG, beta-2 microglobulin; 24hCcr, 24-hour creatinine clearance; U, urine; BSA, body surface area; BUN, blood urea nitrogen
*Only the original Schwartz formula used the value of serum Cr according to the Jaffe method instead of the enzymatic method.

Statistical analysis

All statistical analyses were performed using GraphPad Prism software packages (version 5.0; GraphPad Software, San Diego, CA, USA). The iGFR before and after allo-HSCT was compared using the Wilcoxon's signed-rank sum test to evaluate the impact of allo-HSCT on GFR. The bias of each eGFR was defined as the mean difference between the relevant eGFR and the corresponding iGFR and in percent relative to iGFR [100 × (eGFR–iGFR) / iGFR]. We denoted bias ≥ 10% in any direction as marked bias since median percentage difference of less than 10% of iGFR has been considered clinically acceptable.[35] Bland-Altman's plots were used to display this bias along with the 95% limits of agreement (LOA). It is expected that the 95% LOA include 95% of differences between eGFR and iGFR. The accuracy of each estimating equation compared with the corresponding iGFR was assessed by estimating the proportion of eGFR measurements that were within 10% (P10) and 30% (P30) of the corresponding iGFR measurement. Errors exceeding 30% maybe considered as large, thus 1 − P30 represents the proportion of large errors.[36] P30 accuracy above 75% is considered sufficient for good clinical decision-making but the benchmark is to reach P30 > 90%.[37]

Results

Study population

Of 31 pediatric patients who underwent allo-HSCT during the study period, 17 were enrolled in this study. Of the 14 patients who were excluded from our study, 8 were unable to control their urination and the other 6 did not consent to our study even though they could control their urination. Fourteen participants underwent the iGFR measurement both before and after allo-HSCT, and three underwent the iGFR measurement only once (one because of enrollment in the study after allo-HSCT and two because of refusal to the iGFR measurement after allo-HSCT). We followed and analyzed all 17 participants until either April 2020 or death. Clinical

and laboratory information for the study population is shown in Table 2. The median age of the 17 subjects in the cohort was 10.9 years (4-17 years). Forty-two and forty-eight percent of participants received corticosteroid and/or calcineurin inhibitor (tacrolimus) at the time of the iGFR measurement. Of the 17 participants, 14 received allo-HSCT because of malignancy. All participants received total body irradiation (TBI)-based conditioning, and 11 received myeloablative conditioning (more than 8 Gy of TBI). The iGFR was performed with medians of 13.7 (−19-−11) before allo-HSCT and 73.5 (56-142) after allo-HSCT.

Changes in the inulin glomerular filtration rate after allogeneic stem cell transplantation

The iGFR was not significantly different after allo-HSCT compared with before transplantation (mean iGFR: 105.7 and 105.9 mL/min/1.73 m^2, respectively, $P > 0.999$, Figure 1).

Performance of the estimating equations for the glomerular filtration rate

The measurements of iGFR and performance of the eight methods of calculating eGFR are shown in Table 3. Except for eGFR-CysC CKiD, all estimations exhibited positive bias compared with the corresponding iGFR. The eGFR-β_2MG, eGFR-24hCcr, and eGFR-full CKiD were found to have the small mean bias (9.7%, 8.5% and 8.9%, respectively), which is less than 10%. These biases and the distribution of individual biases are illustrated in Figure 2, which presents Bland-Altman's plots for each eGFR. The solid horizontal lines in Figure 2 are consistent with the values in Table 3, demonstrating the positive bias of each eGFR, except for eGFR-CysC CKiD. The 95% LOA were narrow for eGFR-CysC, eGFR-24hCcr, and eGFR-full CKiD. While eGFR-24hCcr and eGFR-full CKiD exhibited better P10 accuracy in relation to iGFR (41.4% and 48.4%, respectively), eGFR-β_2MG, eGFR-CysC CKiD, eGFR-24hCcr, and eGFR-full CKiD showed good P30 accuracies which were more than 75% (80.6%, 80.6%, 79.3%, and 80.6%, respectively).

Table 2: Participant characteristics

Patient no.	Age	Sex	Disease	Conditioning	Stem cell source	Grade of acute GVHD	Days of iGFR measurement	Height (cm)	Weight (kg)	AMPH-B	Corticosteroid	Calcineurin inhibitor
1	12	Male	Neuroblastoma	L-PAM, Flu, 12Gy TBI	CB	III	-11	142.6	33.3	no	no	no
							65	142.8	29.8	no	yes	yes
2	17	Female	Lymphoma	CY, Flu, 8Gy TBI	CB	II	-11	152.1	59.7	no	no	no
							64	150.5	51.8	no	yes	yes
3	14	Male	ALL	CY, Flu, 8Gy TBI	Related BM	0	-18	170.3	52.1	no	no	no
							58	170.1	49.2	no	yes	yes
4	6	Female	AML	CY, Flu, 8Gy TBI	CB	I	-14	115.5	23.3	no	no	no
							57	115.5	21.5	no	yes	yes
5	7	Male	ALL	CY, Flu, 8Gy TBI	CB	II	-12	121.8	23.7	no	no	no
							63	121.7	20.7	no	yes	yes
6	8	Male	Fanconi anemia	CY, Flu, 3Gy TBI, ATG	Non-related BM	0	-14	122.1	25.8	no	no	no
							57	122.6	21.7	no	no	yes
7*	10	Female	ALL	CY, Flu, 8Gy TBI	CB	NA	-14	133.2	27.5	no	no	no
							NA	NA	NA	NA	NA	NA
8	10	Female	ALL	CY, Flu, 8Gy TBI	CB	I	-19	133.6	23.5	no	no	no
							58	134.8	23.4	no	yes	yes
9	12	Female	ALL	CY, Flu, 8Gy TBI	CB	II	-19	140.6	29.2	no	no	no
							77	141.5	26.1	no	yes	yes

(Continues)

10	12	Female	Aplastic anemia	CY, Flu, 3Gy TBI, ATG	related BM	0	-12	144.0	33.3	no	no	no
							56	144.6	32.7	no	no	yes
11*	15	Female	JMML	CY, Flu, 8Gy TBI	related PBSC	NA	-11	144.2	44.5	no	no	no
							NA	NA	NA	NA	NA	NA
12	10	Male	NK leukemia	CY, Flu, 8Gy TBI	related PBSC	0	-11	141.7	39.7	no	no	no
							58	141.7	40.7	no	no	yes
13	10	Male	ALL	L-PAM, Flu, 4Gy TBI	CB	III	-13	111.2	25.3	no	no	no
							141	113.2	20.4	no	yes	yes
14	15	Female	Lymphoma	CY, Flu, ATG, 8Gy TBI	non-related BM	I	-16	161.6	59.5	no	no	no
							71	162.2	60.5	no	yes	yes
15	4	Male	Chronic granulomatous disease	CY, L-PAM, Flu, ATG, 3Gy TBI	non-related BM	II	-12	99.5	15.7	yes	yes	no
							142	101.1	18.3	no	yes	yes
16	12	Male	ALL	L-PAM, Flu, 3Gy TBI, AraC	non-related BM	I	-12	160.7	48.4	no	no	no
							73	162	41.8	no	yes	yes
17*	12	Male	AML	L-PAM, Flu, 3Gy TBI, AraC	non-related BM	I	NA	NA	NA	NA	NA	NA
							63	158.4	39.55	no	yes	yes

ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; JMML, juvenile myelomonocytic leukemia; L-PAM, melphalan; Flu, fludarabine; TBI, total body irradiation; CY, cyclophosphamide; ATG, anti-thymocyte globulin; CBT, cord blood; BM, bone marrow; PBMC, peripheral blood mononuclear cells; GVHD, graft-versus-host disease; allo-HSCT, allogeneic hematopoietic stem cell transplantation; iGFR, inulin-based glomerular filtration rate; AMPH-B, amphotericin B; NA, not assessed; *, these three participants underwent the iGFR measurement only once.

Table 3: Performance of equations used to estimate the pediatric glomerular filtration rate

Formula	Mean GFR±SD (mL/min/1.73 m^2)	Mean bias		95% LOA (mL/min/1.73 m^2)	10% accuracy (%)	30% accuracy (%)
		Absolute difference (mL/min/1.73 m^2) (95% CI)	Percentage difference (95% CI)			
Inulin-based GFR	105.8±22.8					
Creatinine-based formulas						
Original Schwartz	130.9±19.0	25.1 (14.9 to 35.3)	22.1 (13.5 to 30.6)	-29.5 to 79.7	22.6	51.6
Bedside CKiD	156.2±32.9	50.4 (38.6 to 62.1)	38.0 (29.7 to 46.3)	-12.2 to 112.9	16.1	22.6
Uemura's formula	133.5±29.4	27.7 (16.1 to 39.2)	22.7 (13.8 to 31.7)	-34.1 to 89.4	25.8	54.8
Cystatin C-based formulas						
CKiDcys	89.2±17.4	-16.7 (-24.1 to -9.2)	-16.7 (-24.0 to -9.4)	-56.5 to 23.2	25.8	80.6
Uemura's formula	126.0±28.0	20.2 (10.8 to 29.5)	16.8 (9.0 to 24.7)	-29.7 to 70.0	25.8	61.3
Beta-2 microglobulin-based formula						
Ikezumi's formula	117.7±31.7	11.9 (-0.6 to 24.3)	9.7 (0.2 to 19.3)	-54.5 to 78.3	25.8	80.6
24-hour creatinine clearance-based formula	113.3±24.1	10.2 (2.0 to 18.5)	8.5 (1.2 to 16.3)	-32.2 to 52.7	41.4	79.3
Creatinine- and cystatin C-based formula (full CKiD)	114.9±19.6	9.1 (1.9 to 16.3)	8.9 (2.5 to 15.3)	-29.3 to 47.4	48.4	80.6

GFR, glomerular filtration rate; SD, standard deviation; CI, confidence interval; LOA, limits of agreement; CKiD, chronic kidney disease in children

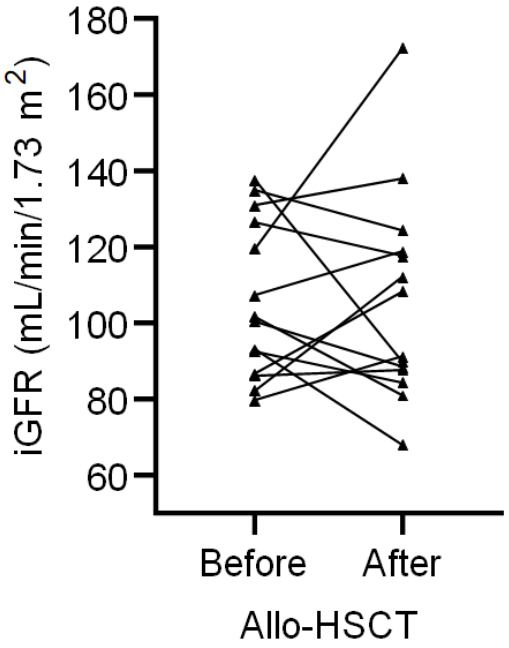

Figure 1 Changes in the inulin glomerular filtration rate in pediatric patients before and after allogeneic stem cell transplantation. There was no significant difference in the inulin-based glomerular filtration rate before and after allogeneic stem cell transplantation (mean value: 105.7 and 105.9 mL/min/1.73 m^2, respectively). There was one case of chronic kidney disease. Abbreviations: allo-HSCT, allogeneic stem

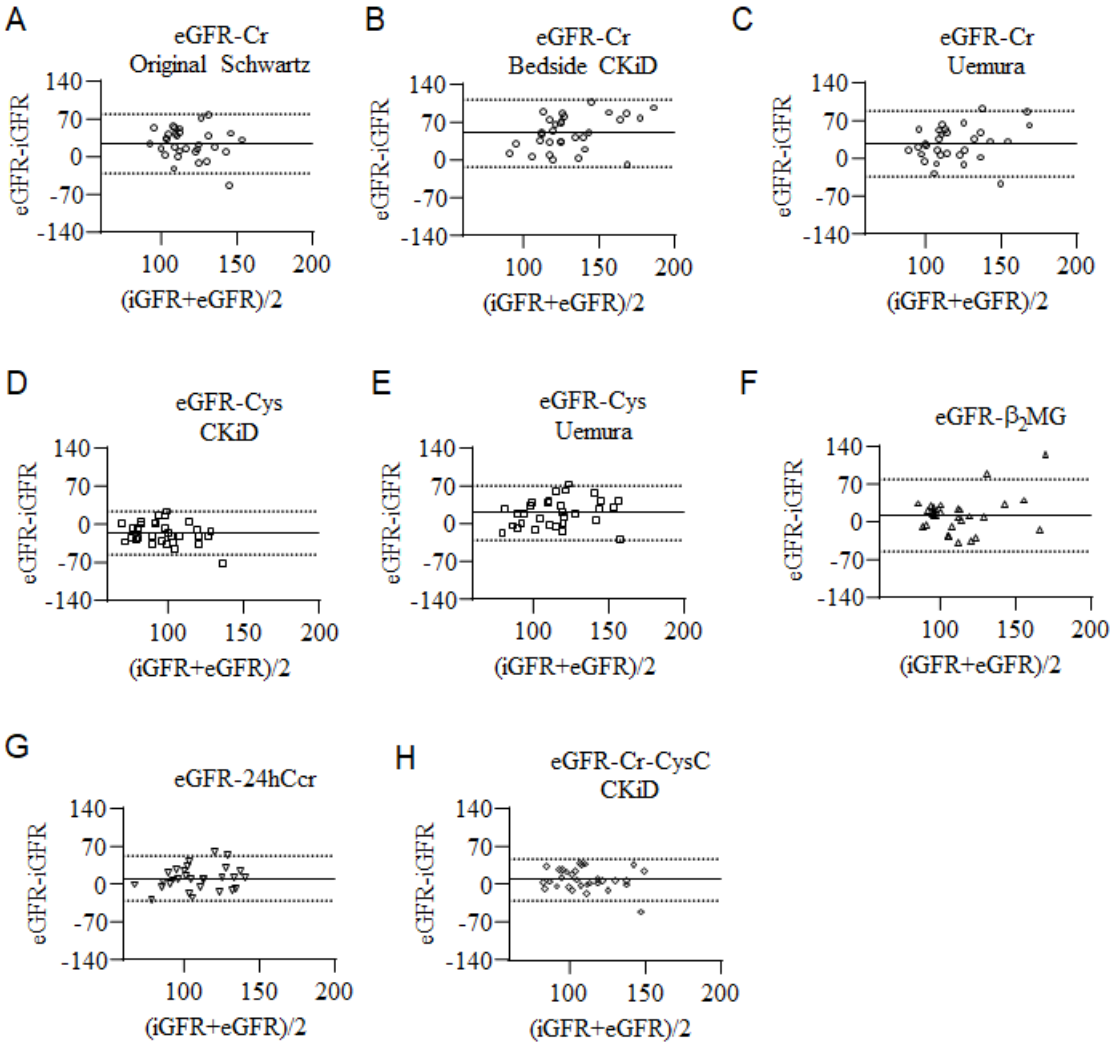

Figure 2 Bland-Altman's plots for each estimated glomerular filtration rate relative to the corresponding inulin-based glomerular filtration rate.
Solid horizontal lines represent the mean difference between estimated and inulin-based glomerular filtration rates (bias), and dashed horizontal lines represent 95% limits of agreement. Except for the estimated glomerular rate calculated using cystatin C in the Chronic Kidney Disease in Children formula, all rates exhibit positive bias compared with the corresponding inulin-based glomerular filtration rate. Abbreviations: β_2MG, beta-2 microglobulin; CKiD, Chronic Kidney Disease in Children; Cr, creatinine; Cys, cystatin C; eGFR, estimated glomerular filtration rate; iGFR, inulin-based glomerular filtration rate.

Discussion

Although accurate assessment of kidney function is essential following allo-HSCT, there have been no reports assessing iGFR, which is regarded as the gold standard measurement of GFR, in pediatric patients undergoing HSCT. This study is the first report to assess formal GFR using renal clearance of inulin before and after allo-HSCT in pediatric patients. There was no significant impact of allo-HSCT on GFR in our cohort, showing that the impact of allo-HSCT on kidney function at early time point after allo-HSCT was slight. However, we cannot draw conclusions about the development of CKD from our study because we assessed iGFR after allo-HSCT at a median days of 73.5 after allo-HSCT. To assess develop of CKD in our cohort, periodic long follow up will be needed.

Because GFR estimating equations are based on the results of direct measurements and are constructed to match the measured GFR, which serves as the independent variable during the statistical modeling, eGFR equations are heavily dependent on the data used during the development of the equation.[3] Therefore, equations based on data from children with HSCT will differ from equations based on data from children with a diseased kidney, suggesting that assessment of the performance of each eGFR in pediatric patients with allo-HSCT will be useful for clinical practice. Our evaluation of the performance of common equations for estimating GFR in children revealed that those relying on serum Cr (original Schwartz formula, bedside CKiD formula, and Uemura's formula) have large positive bias and large 95% LOA, even though Uemura's formula was specifically developed for assessment of eGFR in Japanese children. The results that we obtained using the original Schwartz and bedside CKiD formulae are consistent with those of previous reports.[8,38] Thus, calculation of eGFR using the serum Cr concentration may not be suitable for pediatric patients undergoing allo-HSCT. Many factors affect Cr generation including body mass, body size, diet, nutritional status, and muscle mass.[2,14,15] The accuracy of eGFR-Cr may be reduced, especially in children with allo-HSCT, due to the effects of long-term hospitalization, use of glucocorticoids, conditioning, GVHD,

and so on. Because eGFR-24hCcr is used frequently in the case of hospitalized pediatric patients who can control their urination and do not have nocturnal enuresis, we also evaluated the performance of this approach and found it to be a reliable method. Estimation of eGFR using 24-hCcr showed good accuracy, small mean bias, and small 95% LOA compared with the corresponding iGFR (Table 3 and Figure 2). However, eGFR-24hCcr is not practical especially in outpatients, babies, and infants because a long time is required for urine collection, and patients with nocturnal enuresis cannot be assessed using this method. Other than eGFR-24hCcr, eGFR-full CKiD which include Cr, CysC, and BUN is a promising method of eGFR, because eGFR-full CKiD also has good accuracy (both P10 and P30), small mean bias, and small 95% LOA compared with the corresponding iGFR. Taking the simplicity of the measurement, we suggest that eGFR-full CKiD is a favorable method for evaluating eGFR in pediatric patients undergoing allo-HSCT in clinical practice. Ikezumi et al. reported that the β_2MG-based eGFR formula is useful for clinical screening of renal function in Japanese children and adolescents, especially in cases where severe muscle loss has occurred[20]; however, we found the reliability of this method to be very poor because of its large 95% LOA. Serum β_2MG levels are increased in patients with not only kidney dysfunction, but also several malignancies, infectious diseases, inflammation, and lymphoproliferative disorders.[39-45] Because many factors affect serum β_2MG levels in allo-HSCT, such as infection and GVHD, we supposed that serum β_2MG levels changed easily.

The cost-effectiveness is also important factor in clinical practice. While eGFR-full CKiD calculated with serum Cr, CysC, and BUN is better marker for renal function than that of eGFR-Cr, the cost of their measurements is about ten-fold expensive than that of serum Cr alone. Thus, we need to consider carefully the time point of eGFR-full CKiD measurement before and after allo-HSCT. On the other hand, because our results show that eGFR-Cr is not reliable method for estimation of GFR before and after allo-HSCT in pediatric patients, eGFR-full

CKiD should be assessed periodically to detect decline of kidney function, for example, before allo-HSCT, at the time of discharge from hospital, and an elevation of serum Cr more than 1.5 times or 0.3 mg/dl from baseline (Stage 1 AKI by the Kidney Disease Improving Global Outcomes[46]).

Our study has several limitations that should be acknowledged. Firstly, we could not assess the effect of confounders, such as corticosteroid, amphotericin B, and calcineurin inhibitor on eGFR reliability because the cohort was relatively small. Funakoshi et al. reported that the reliability of eGFR was deteriorated by cisplatin treatment, and therefore different equations need to be considered after administration of specific treatment.[47] A larger sample size is needed to assess the reliability of eGFR before and after allo-HSCT. Secondly, we could not assess the utility of eGFR in relation to patients' deterioration of kidney function status, because we observed only one patient who had deterioration of iGFR at the time of the iGFR measurement (patient no. 13, iGFR 68.14 mL/min/1.73 m^2). Further evaluation, of both iGFR and eGFR in children who have CKD before and after allo-HSCT is required to assess the utility of eGFR in such patients. Finally, we were unable to carry out a comprehensive assessment of the influence of chronic GVHD, which has been reported to affect kidney function,[48-51] because iGFR after allo-HSCT was taken as the iGFR at the time of discharge; it is possible that chronic GVHD may develop after this time. Therefore, we could not assess CKD in our cohort. Evaluating iGFR at a later time point after allo-HSCT, for example at 1 year, may offer some insight into this.

In conclusion, the impact of allo-HSCT at early time point on GFR using renal clearance of inulin was not significant in our cohort. The most reliable equations of GFR for pediatric patients with allo-HSCT were eGFR-24hCcr and eGFR-full CKiD; however, eGFR-full CKiD seems to be a more useful method because of the simplicity of measurement.

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