High bias and low precision for estimated *versus* measured glomerular filtration rate in pediatric sickle cell anemia

Individuals with sickle cell anemia (SCA) develop glomerular injury that progresses to chronic kidney disease.[1] Measured GFR (mGFR) is the gold standard test for monitoring glomerular function. Pediatric SCA research has used ^{99m}Tc-DTPA to determine the mGFR but this test is not feasible for annual monitoring because of its high cost and time commitment.[2] A more convenient approach to track glomerular function is to measure serum creatinine (SCr) and/or cystatin C (CyC) and calculate the estimated glomerular filtration rate (eGFR). Several pediatric eGFR equations were validated in non-SCA populations; however, each of these equations has limitations and none has been validated in SCA.[3-7]

It is imperative to identify the eGFR equation with the least bias of eGFR relative to mGFR and highest precision for SCA clinical care and research. Bias defines the accuracy (eGFR minus mGFR) and standard deviation of this bias defines the precision of eGFR. The Institutional Review Board-approved Sickle Cell Clinical Research and Intervention Program (SCCRIP) requires annual eGFR and mGFR using ^{99m}Tc-DTPA every 3 to 6 years (NCT:020988635).[8] To determine the accuracy and precision of five pediatric eGFR equations, we tested the hypotheses that: (i) in comparison to mGFR, estimates using the Chronic Kidney Disease in Children (CKiD) eGFR equation would have lowest bias and highest precision; (ii) bias would be similar by therapy and age; and (iii) the intrapatient variability for the bias would be low among patients with repeated measures.

Among patients with eGFR and mGFR obtained within a 4-week period enrolled in the SCCRIP study, we performed an agreement analysis between five standard pediatric eGFR equations derived from either SCr, SCr and blood urea nitrogen (BUN), CyC, or SCr and CyC and mGFR by ^{99m}Tc-DTPA clearance.[5-7] We excluded five participants with either chronic kidney disease or severe hyperfiltration (mGFR: <60 or >240, eGFR: >350 mL/min/1.73m^2). We determined eGFR and mGFR at outpatient appointments during clinician-determined steady state. We compared the bias (eGFR-mGFR) in patients with repeated measures and categorized patients as having low intrapatient variability if the absolute difference in the bias on repeated measures was <5 mL/min/1.73m^2.

Summary statistics including mean and standard deviation (SD) for continuous variables and counts and percentages for categorical variables were reported and compared using statistical tests as appropriate. For the agreement analyses between mGFR and eGFR, mean bias (95% limits of agreement) and the SD of bias were calculated using Bland-Altman methods assuming constant variance. A *t*-test and F-test were used to compare bias and variance between the CKiD equation and four other pediatric eGFR equations. The Pearson correlation (r) or Spearman rank correlation (ρ), and Lin concordance correlation (CCC) with 95% confidence intervals are presented. The percentage difference between eGFR and mGFR and the percentage of values within ±10% (P10[%]) and ±30% (P30[%]) are presented. A linear regression model was used to assess the effects of age, treatments and/or their interaction on bias between eGFR and mGFR. Next, we analyzed patients with repeated measurements. We performed generalized least squares models to assess the effects of age, treatments and/or their interaction on bias. We modeled the correlations of repeated measurements on the same subjects using a compound symmetry correlation structure. All *P*-values were two-sided and considered statistically significant when <0.05. Analyses were performed using SAS v9.4 (Cary, NC, USA) and R-3.5.2 (Vienna, Austria).

Three-hundred sixty-four ^{99m}Tc-DTPA mGFR examinations were performed in 198 subjects. Among the 198 individuals with an initial mGFR, 196 also had SCr and 124 had SCr and CyC measured within 4 weeks of mGFR. The median age of the 198 participants at the time of their initial mGFR was 8.2 years (range, 2.1-18.0). The mean (± SD) mGFR was 141± 26 mL/min/1.73 m^2. Eighty-nine (45%) participants were female. No difference was observed in the mean age of female and male participants (8.6 *vs.* 8.5 years, *P*=0.83). However, the mean mGFR (± SD) was significantly higher in males than females (145±26 *vs.* 137±26 mL/min/1.73 m^2, *P*=0.024). At the time of mGFR, participants were receiv-

Table 1. Bias, precision, agreement and accuracy of the estimated compared to glomerular filtration rate (GFR) compared to ^{99m}Tc-DTPA measurements of GFR.

eGFR equation	N	Bias (95% limits of agreement) mGFR-eGFR	SD of bias	CCC	Correlation coefficient (ρ or r)	P10 (%)	P30 (%)
Pediatric creatinine-based equations							
Schwartz creatinine-based equation (2009)	196	-21.4 (-80, 37.2)*	29.9#	0.38 (0.28, 0.47)	ρ=0.49[a]	34.7%	69.9%
Schwartz creatinine BUN- based equation (2009)	196	10.7 (-36.4, 57.8)*	24.02#	0.44 (0.33, 0.53)	r=0.50	41.3%	92.9%
Pediatric cystatin-based equations							
Schwartz cystatin C-based equation (2012)	126	47.7 (-1.8, 97.2)*	25.3#	0.08 (0.04, 0.12)	ρ=0.36[a]	4.8%	36.3%
Filler equation (2003)	126	12.5 (-43.1, 68.1)	28.4#	0.3 (0.15, 0.44)	ρ=0.36[a]	26.6%	87.1%
Pediatric creatinine-cystatin equation							
Pediatric CKiD creatinine-cystatin equation (2012)	124	17.0 (-22.0, 55.9)	19.86	0.44 (0.34, 0.53)	r=0.66	41.9%	95.2%

Schwartz creatinine-based equation: eGFR= 41.3 x (height (m)/SCr); Schwartz creatinine/BUN-based equation: eGFR= 40.7 x (height (m)/SCr)$^{0.64}$x(30/BUN)$^{0.202}$; Schwartz cystatin C-based equation: eGFR=70.69 x (SCysC)$^{-0.931}$; Filler cystatin C equation: $\log_e$(GFR) = 1.962 + [1.123 × $\log_e$ (1/CysC)]; CKiD equation: GFR=39.8 x [height/SCr]$^{0.456}$ x [1.8/CysC]$^{0.418}$ x [30/BUN]$^{0.079}$ x [1.076^{male}] [1.00 ^{female}] x [height (m)/1.4]0.179 *P-value <0.05 for the comparison of bias in the other equations to that in the Pediatric CKiD creatinine-cystatin equation based on a two-sided *t*-test. #P-value <0.05 for the comparison of variance in bias in the other equations to that in the Pediatric CKiD creatinine-cystatin equation based on a two-sided F-test. a their Pearson correlation coefficients (r) are almost identical to ρ. N: number; mGFR: measured glomerular filtration rate; eGFR: estimated glomerular filtration rate; SD; standard deviation; SD: standard deviation, CCC: Lin concordance correlation coefficient, ρ: Spearman rank correlation, r: Pearson correlation, P10%: percentage of eGFR values within ±10% of GFR; P30%: percentage of eGFR values within ±30% of GFR; SCr: serum creatinine; CysC: cystatin C; BUN: blood urea nitrogen; CKiD; Chronic Kidney Disease In Children.

ing either hydroxyurea alone (n=73), hydroxyurea and chronic transfusion therapy (n=17), chronic transfusion therapy alone (n=12), or no SCA-modifying therapy (n=96). We present the mean bias, precision (SD of bias), and agreement (Lin concordance correlation, Pearson/Spearman rank correlation, P10 and P30) of the fit for mGFR as compared to five eGFR equations. (Table 1, Figure 1). The Filler CyC or the Schwartz SCr/BUN eGFR equations had the smallest mean bias. The CKID eGFR equation had the lowest SD, highest correlation (r=0.66), concordance correlation (0.44), and P30. We compared the bias in four eGFR equations to the that of the CKiD equation; the CKiD equation had significantly lower bias than the Schwartz SCr equation ($P=2\times10^{-22}$) but did not have statistically lower bias than the other three eGFR equations. (Table 1, Figure 1). The CKiD equation had a statistically significant lower SD than all other equations ($P<0.05$).

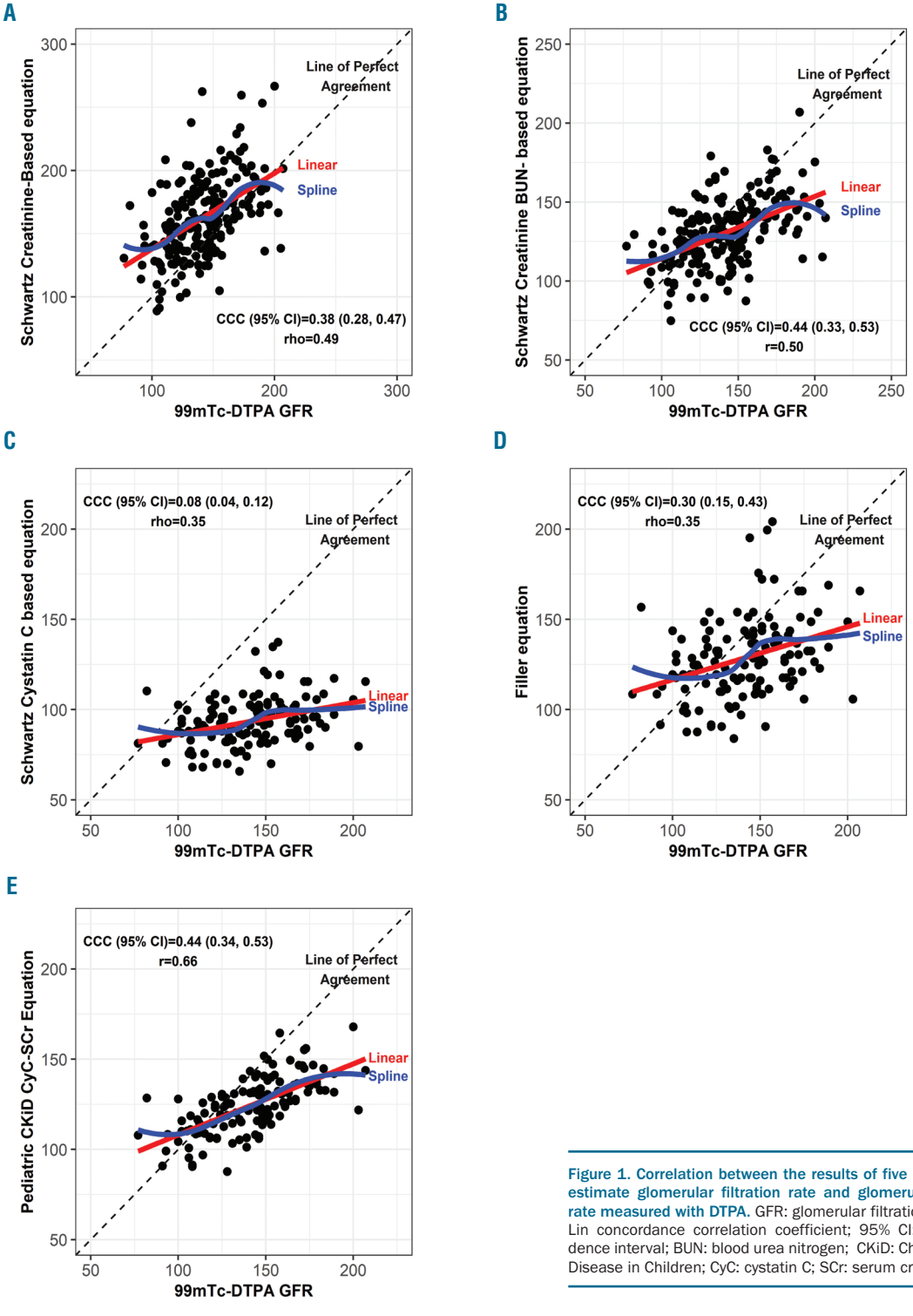

Figure 1. Correlation between the results of five equations to estimate glomerular filtration rate and glomerular filtration rate measured with DTPA. GFR: glomerular filtration rate; CCC: Lin concordance correlation coefficient; 95% CI: 95% confidence interval; BUN: blood urea nitrogen; CKiD: Chronic Kidney Disease in Children; CyC: cystatin C; SCr: serum creatinine.

Table 2. Change in measured glomerular filtration rate (mGFR) on repeat evaluation *versus* change in estimated glomerular filtration rate (eGFR) equations on repeat evaluation and number (%) of participants with high accuracy of repeat change. ($mGFR_{repeat}$ – $mGFR_{baseline}$) – ($eGFR_{repeat}$ – $eGFR_{baseline}$).

	Δ mGFR *vs.* Δ Schwartz Cr	Δ mGFR *vs.* Δ Schwartz BUN Cr	Δ mGFR *vs.* Δ Schwartz CyC	Δ mGFR *vs.* Δ Filler CyC	Δ mGFR *vs.* Δ CKiD CyC & Cr
Number	103	103	44	44	42
Difference in the change in mGFR *vs.* eGFR on repeat evaluation (mL/min/1.73 m^2), Mean (SD)*	-13.8 (20.4)	15.3 (18.5)	45.3 (13.8)	10.5 (16.1)	18.0 (13.0)
Number and % of patients with intrasubject Δ bias† <5 mL/min/1.73 m^2	6 (5.8%)	6 (5.8%)	7 (15.9%)	6 (13.6%)	7 (16.7%)
Number and % of patients with intrasubject Δ bias† <10 mL/min/1.73 m^2	18 (17.5%)	21 (20.4%)	17 (40.5%)	12 (27.3%)	17 (40.5%)

*Mean (SD) of intrasubject bias estimates. †intrasubject Δ bias calculated as range of bias estimates. Δ mGFR; $mGFR_{repeat}$ – $mGFR_{baseline}$). Δ eGFR ($eGFR_{repeat}$ – $eGFR_{baseline}$). Cr: creatinine; BUN; blood urea nitrogen; CysC: cystatin C; CKiD: Chronic Kidney Disease in Children; SD: standard deviation.

We sought to investigate the association of age and therapy on the bias. Age was not significantly associated with bias except when using the Schwartz SCr equation (Schwartz SCr *vs.* mGFR *P*=0.005; other *P*-values: 0.3-0.9). Therapy was not significantly associated with bias between mGFR or any of the five eGFR equations (range of *P*-values: 0.2-0.6). Finally, we did not identify an association of age with bias that was modified by hydroxyurea therapy for any of the five eGFR equations.

We analyzed the intrapatient variability in the bias among participants who had initial and at least one additional mGFR and eGFR measurements. Using all five eGFR equations, the mean difference in the bias between the initial mGFR and eGFR and the bias on the repeat evaluation for individuals ranged from 10.5 mL/min/1.73m^2 (using the Filler CyC equation) to 45.3 mL/min/1.73m^2 (using the Schwartz CyC equation) (Table 2). No eGFR equation identified more than 20% of patients with a change in bias from baseline that was within 5 mL/min/1.73m^2 of the bias on the repeated measures.

Our findings show that the Schwartz SCr/BUN (10.7 mL/min/1.73m^2) and the Filler CyC (12.5 mL/min/1.73m^2) equations had the lowest bias while the CKiD equation had the highest correlation (0.66). In comparison, the CKiD equation was developed and validated with a bias of -0.2 mL/min/1.73m^2 and a correlation of 0.92.5 This comparison highlights the limitations of using the current eGFR equations as validated in pediatric SCA research and clinical care. Despite the overall limitation in the bias and precision, our data suggest that clinicians monitoring annual eGFR should obtain SCr and CyC to decrease the bias and imprecision of SCr-alone eGFR equations. Our data were limited to children; additional research is needed in adult SCA.[9] In one recent study in SCA adults (n=12) comparing mGFR (determined using iohexol) and eGFR equations, the eGFR calculated by CyC alone again had the lowest bias (0.2 mL/min/1.73 m^2) but high imprecision (SD 26.3 mL/min/1.73 m^2).[10]

A second important finding of our study is that high intrapatient variability in the bias was observed using repeated evaluations. It is well established that systematic bias exists between mGFR and eGFR.[9,11] Researchers may accept this bias if it is consistent on repeated measures. The CKiD study demonstrated this low intrapatient variability on repeated measures; the annual change in the bias from baseline (mGFR-eGFR) to annual follow-up (mGFR-eGFR) was approximately 1 mL/min/1.73 m^2.[12] Our data using the CKiD equation identified a mean difference in the bias on repeat evaluations of 18 mL/min/1.73 m^2. This high intrapatient variability in the bias on repeat evaluations should preclude assumptions in a pediatric SCA clinical trial using renoprotective agents that eGFR changes from baseline to exit confirm the magnitude or direction of changes in mGFR.

Next, this study identifies sex differences in mGFR in pediatric SCA. The mGFR in males was significantly higher than in females, which replicates SCA murine data that male mice develop a higher mGFR than females.[13] A GFR difference by sex has not been well studied in SCA; however, some adult SCA studies have found that male patients with chronic kidney disease have a greater annual decline in eGFR, increased risk of acute kidney injury, and a higher mortality rate as compared to females.[14,15] Next, age did not influence the bias between mGFR in four of the five eGFR equations and hydroxyurea therapy did not affect the bias between mGFR and five eGFR equations.

Several novel findings relevant to clinicians and researchers emerge from this study; however, some limitations are worth noting. First, CyC and SCr levels were accepted if performed within 4 weeks of mGFR. Second, adult mGFR data were not available. Therefore, future prospective research should perform all tests on the same day and include high-risk adult participants with eGFR <60 mL/min/1.73 m^2.

In conclusion, we demonstrate that for pediatric clinical care, annual eGFR equations should include SCr and CyC although recognizing the limitations of this approach. For natural history studies, we suggest using mean eGFR over several time points to minimize imprecision. Pediatric trials of novel renoprotective therapies should use mGFR. There is an urgent need to develop either a more precise eGFR equation validated for SCA or an efficient, economical mGFR method. Validated tests of glomerular function are essential to reduce the morbidity and mortality associated with SCA kidney disease.

Jeffrey D. Lebensburger,[1] Jeffrey Gossett,[2] Rima Zahr,[3] Winfred C. Wang,[4] Kenneth I. Ataga,[5] Jeremie H. Estepp,[4] Guolian Kang[2] and Jane S. Hankins[4]

[1]Department of Pediatrics, University of Alabama, Birmingham, AL; [2]Department of Biostatistics, St. Jude Children's Research Hospital, University of Alabama at Birmingham, Birmingham, AL; [3]Division of Pediatric Nephrology and Hypertension, University of Tennessee Health Science Center, Memphis, TN; [4]Department of Hematology, St. Jude Children's Research Hospital, Memphis TN and [5]Center for

Sickle Cell Disease, University of Tennessee Health Science Center, Memphis, TN, USA

Correspondence:
JEFFREY D. LEBENSBURGER - jlebensburger@peds.uab.edu

doi:10.3324/haematol.2019.242156

Disclosures: JDL is a consultant for Novartis and has received funding from Pfizer ASPIRE. JHE receives research support from Pfizer and Eli Lilly and Co.and serves as a consultant for Daiichi Sankyo and Global BloodTherapeutics; WCW receives research support from Novartis and Agios; JSH receives research support from Global Blood Therapeutics; KIA is a member of an advisory board for Novartis, Global Blood Therapeutics, Novo Nordisk, Editas Medicine, and Bioverativ.

Contributions: JDL and JSH developed the concept and wrote the manuscript. JG and GK performed the data analysis. JHE, KIA, WCW, RZ assisted in the study design and manuscript preparation.

References

1. Sharpe CC, Thein SL. How I treat renal complications in sickle cell disease. Blood. 2014;123(24):3720-3726.
2. Wang WC, Ware RE, Miller ST, et al. Hydroxycarbamide in very young children with sickle-cell anaemia: a multicentre, randomised, controlled trial (BABY HUG). Lancet. 2011;377(9778):1663-1672.
3. Aygun B, Mortier NA, Smeltzer MP, Hankins JS, Ware RE. Glomerular hyperfiltration and albuminuria in children with sickle cell anemia. Pediatr Nephrol. 2011;26(8):1285-1290.
4. McPherson Yee M, Jabbar SF, Osunkwo I, et al. Chronic kidney disease and albuminuria in children with sickle cell disease. Clin J Am Soc Nephrol. 2011;6(11):2628-2633.
5. Schwartz GJ, Schneider MF, Maier PS, et al. Improved equations estimating GFR in children with chronic kidney disease using an immunonephelometric determination of cystatin C. Kidney Int. 2012;82(4):445-453.
6. Filler G, Lepage N. Should the Schwartz formula for estimation of GFR be replaced by cystatin C formula? Pediatr Nephrol. 2003;18(10):981-985.
7. Schwartz GJ, Munoz A, Schneider MF, et al. New equations to estimate GFR in children with CKD. J Am Soc Nephrol. 2009;20(3):629-637.
8. Hankins JS, Estepp JH, Hodges JR, et al. Sickle Cell Clinical Research and Intervention Program (SCCRIP): a lifespan cohort study for sickle cell disease progression from the pediatric stage into adulthood. Pediatr Blood Cancer. 2018;65(9):e27228.
9. Asnani M, Reid M. Cystatin C: a useful marker of glomerulopathy in sickle cell disease? Blood Cells Mol Dis. 2015;54(1):65-70.
10. Yee ME, Lane PA, Archer DR, Joiner CH, Eckman JR, Guasch A. Losartan therapy decreases albuminuria with stable glomerular filtration and permselectivity in sickle cell anemia. Blood Cells Mol Dis. 2018;69:65-70.
11. Yee MEM, Lane PA, Archer DR, Joiner CH, Eckman JR, Guasch A. Estimation of glomerular filtration rate using serum cystatin C and creatinine in adults with sickle cell anemia. Am J Hematol. 2017;92(10):E598-E599.
12. Ng DK, Schwartz GJ, Warady BA, Furth SL, Munoz A. Relationships of measured iohexol GFR and estimated GFR with CKD-related biomarkers in children and adolescents. Am J Kidney Dis. 2017;70(3):397-405.
13. Kasztan M, Fox BM, Lebensburger JD, et al. Hyperfiltration predicts long-term renal outcomes in humanized sickle cell mice. Blood Adv. 2019;3(9):1460-1475.
14. Stallworth JR, Tripathi A, Jerrell JM. Prevalence, treatment, and outcomes of renal conditions in pediatric sickle cell disease. Southern Med J. 2011;104(11):752-756.
15. McClellan AC, Luthi JC, Lynch JR, et al. High one year mortality in adults with sickle cell disease and end-stage renal disease. Br J Haematol. 2012;159(3):360-367.