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

by Jeffrey D. Lebensburger, Jeffrey Gossett, Rima Zahr, Winfred C. Wang, Kenneth I. Ataga, Jeremie H. Estepp, Guolian Kang, and Jane S. Hankins

Haematologica 2020 [Epub ahead of print]

Citation: Jeffrey D. Lebensburger, Jeffrey Gossett, Rima Zahr, Winfred C. Wang, Kenneth I. Ataga, Jeremie H. Estepp, Guolian Kang, and Jane S. Hankins. High bias and low precision for estimated versus measured glomerular filtration rate in pediatric sickle cell anemia.
Haematologica. 2020; 105:xxx
doi:10.3324/haematol.2019.242156

Publisher's Disclaimer.
E-publishing ahead of print is increasingly important for the rapid dissemination of science. Haematologica is, therefore, E-publishing PDF files of an early version of manuscripts that have completed a regular peer review and have been accepted for publication. E-publishing of this PDF file has been approved by the authors. After having E-published Ahead of Print, manuscripts will then undergo technical and English editing, typesetting, proof correction and be presented for the authors' final approval; the final version of the manuscript will then appear in print on a regular issue of the journal. All legal disclaimers that apply to the journal also pertain to this production process.
High bias and low precision for estimated versus measured glomerular filtration rate in pediatric sickle cell anemia

Short Title: mGFR vs eGFR in Pediatric SCA

Jeffrey D. Lebensburger¹, Jeffrey Gossett², Rima Zahr³, Winfred C. Wang⁴, Kenneth I. Ataga⁵, Jeremie H. Estepp⁴, Guolian Kang², Jane S. Hankins⁴

¹. Department of Pediatrics, University of Alabama at Birmingham, 2. Department of Biostatistics, St. Jude Children’s Research Hospital 3. Division of Pediatric Nephrology and Hypertension, University of Tennessee Health Science Center at Memphis 4. Department of Hematology, St. Jude Children’s Research Hospital. 5. Center for Sickle Cell Disease, University of Tennessee Health Science Center at Memphis.

Corresponding Author: Jeffrey Lebensburger DO, MSPH. 1600 7th Ave. S. 512 Lowder. Birmingham AL 35223. jlebensburger@peds.uab.edu. Phone 205 638-9285.

Text Word Count: 1498

Figures: 1

Tables: 2

References: 15
Sickle cell anemia (SCA) individuals develop glomerular injury that progresses to chronic kidney disease (CKD).(1) Measured GFR (mGFR) is the gold standard test for monitoring glomerular function. Pediatric SCA research has used $^{99m}$Tc-DTPA for mGFR but this test is not feasible for annual monitoring due to 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, limitations exist with each of these equations and they have not 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 IRB-approved Sickle Cell Clinical Research and Intervention Program (SCCRIPT) performs annual eGFR and every three to six years mGFR using $^{99m}$Tc-DTPA (NCT:020988635).(8) To determine the accuracy and precision of five pediatric eGFR equations, we tested the hypotheses that 1) in comparison to mGFR, estimations using the CKID eGFR equation would have lowest bias and highest precision, 2) bias would be similar by therapy and age, and 3) the intrapatient variability for the bias would be low among patients with repeated measures.

Among patients with eGFR and mGFR obtained within a four-week period enrolled in the SCCRIP study, we performed an agreement analysis between five standard pediatric eGFR equations derived from either SCr, SCr and BUN, CyC, or SCr and CyC and mGFR by $^{99m}$Tc-DTPA clearance.(5-7) We excluded five participants with either CKD or severe hyperfiltration (mGFR: <60 or >240, eGFR: >350mL/min/1.73m$^2$). We performed outpatient eGFR and mGFR 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 $<5\text{mL/min/1.73m}^2$.

Summary statistics including mean and standard deviation (s.d.) 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 s.d. of bias were calculated using Bland-Altman methods assuming constant variance. 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’s rank correlation ($\rho$), and Lin’s concordance correlation (CCC) with 95% confidence intervals are presented. The percentage difference between eGFR and mGFR and the percentage of values within $\pm10\%$ [P10(%)$]$ and $\pm30\%$ [P30(%)$]$ are presented. Linear regression model was used to assess the effect 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 $p < 0.05$ was considered significant. Analyses were performed using SAS v9.4 (Cary, NC) and R-3.5.2 (Vienna, Austria).

Three hundred and sixty four $^{99}$Tc-DTPA mGFR examinations were performed in 198 subjects. Among the 198 individuals with an initial mGFR, 196 individuals also had a SCr and 124 had a SCr and a 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 ($\pm$ s.d.) mGFR was 141±
26 mL/min/1.73 m². 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 (± s.d.) was significantly higher in males than females (145±26 vs. 137±26 mL/min/1.73 m², p=0.024). At the time of mGFR, participants were receiving 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 (s.d. of bias), and agreement (Lin’s concordance correlation, Pearson/Spearman’s 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 s.d., highest correlation (r=0.66), concordance correlation (0.44), and P30. We compared the bias in four eGFR equations to the CKiD equation; the CKiD equation had significantly lower bias than the Schwartz SCr equation (p=2×10⁻²²) 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 s.d. than all other equations (p<0.05).

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 range 0.3-0.9). Therapy was not significantly associated with bias between mGFR nor 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 all five eGFR equations.
We analyzed the intrapatient variability in the bias among participants who had an 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 5mL/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 pediatrics; additional research is needed in adult SCA.(9) In one recent adult SCA study (n=12) comparing mGFR (iohexol) to eGFR equations, the eGFR calculated by CyC alone again had the lowest bias (0.2 mL/min/m$^2$) but high imprecision (s.d. 26.3 mL/min/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 m². (12) Our data using the CKID equation identified a mean difference in the bias on repeat evaluations of 18 mL/min/1.73 m². 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 females which replicate SCA murine data that male mice develop a higher mGFR than females. (13) GFR difference by sex has not been well studied in SCA; however, some adult SCA studies have found that male patients with CKD have a higher annual decline in eGFR, increased risk for 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 impact the bias between mGFR and five eGFR equations.

This study identifies several novel findings relevant to clinicians and researchers; however, some limitations are worth noting. First, CyC and SCr levels were accepted if performed within four 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 m².

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 the
imprecision. Pediatric trials of novel renoprotective therapies should use mGFR. There is an
urgent need to either develop a more precise eGFR equation validated for SCA or develop an
efficient, economical mGFR method. Validated tests of glomerular function are essential to
reduce the morbidity and mortality associated with SCA kidney disease.
Acknowledgements:

Authorship 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.

Conflict of Interest Disclosures:

JDL is a consultant for Novartis and has received funding from Pfizer ASPIRE.

J.H.E. receives research support from Pfizer and Eli Lilly and Co. and serves as a consultant for Daiichi Sankyo and Global Blood Therapeutics.

W.C.W. receives research support from Novartis and Agios.

J.S.H. 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.

Table 1: Bias, precision, agreement and accuracy of the estimated GFR compared to $^{99m}$Tc-DTPA measurements of GFR.

<table>
<thead>
<tr>
<th>eGFR equation</th>
<th>N</th>
<th>Bias (95% limits of agreement)</th>
<th>SD of bias</th>
<th>CCC</th>
<th>Correlation Coefficient ($\rho$ or $r$)</th>
<th>P10 (%)</th>
<th>P30 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pediatric Creatinine-based equations</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Schwartz Creatinine-Based equation (2009)</td>
<td>196</td>
<td>-21.4 (-80, 37.2)*</td>
<td>29.9#</td>
<td>0.38 (0.28, 0.47)</td>
<td>$\rho=0.49^a$</td>
<td>34.7%</td>
<td>69.9%</td>
</tr>
<tr>
<td>Schwartz Creatinine BUN-based equation (2009)</td>
<td>196</td>
<td>10.7 (-36.4, 57.8)*</td>
<td>24.02#</td>
<td>0.44 (0.33, 0.53)</td>
<td>$r=0.50$</td>
<td>41.3%</td>
<td>92.9%</td>
</tr>
<tr>
<td><strong>Pediatric Cystatin-based equations</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Schwartz Cystatin C based equation (2012)</td>
<td>126</td>
<td>47.7 (-1.8, 97.2)*</td>
<td>25.3#</td>
<td>0.08 (0.04, 0.12)</td>
<td>$\rho=0.36^a$</td>
<td>4.8%</td>
<td>36.3%</td>
</tr>
<tr>
<td>Filler Equation (2003)</td>
<td>126</td>
<td>12.5 (-43.1, 68.1)</td>
<td>28.4#</td>
<td>0.3 (0.15, 0.44)</td>
<td>$\rho=0.36^a$</td>
<td>26.6%</td>
<td>87.1%</td>
</tr>
<tr>
<td><strong>Pediatric Creatinine-Cystatin Equation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pediatric CKID Creatinine-Cystatin Equation (2012)</td>
<td>124</td>
<td>17.0 (-22.0, 55.9)</td>
<td>19.86</td>
<td>0.44 (0.34, 0.53)</td>
<td>$r=0.66$</td>
<td>41.9%</td>
<td>95.2%</td>
</tr>
</tbody>
</table>

Schwartz Creatine Based Equation: eGFR= 41.3 x (height (m)/Scrn)
Schwartz Creatinine/BUN Based Equation: eGFR= 40.7 x (height (m)/Scrn)$^{0.64}$ x (30/BUN)$^{0.202}$
Schwartz Cystatin C Based Equation: eGFR=70.69 x (ScysC)$^{-0.931}$
Filler Cystatin C Equation: Log10(GFR) = 1.962+[1.123 x log10 (1/Cystatin C)]
CKID: GFR=39.8 x [ht/Scrn]$^{0.456}$ x [1.8/cysC]$^{0.418}$ x [30/BUN]$^{0.079}$ x [1.076$^{\text{male}}$] x [1.00$^{\text{female}}$] x [ht (m)/1.4]$^{0.179}$

SD: standard deviation, CCC: Lin’s concordance correlation coefficient, $\rho$: Spearman’s rank correlation, $r$: Pearson correlation, P10% is the percentage of eGFR values within +/-10% of GFR. P30% is the percentage of eGFR values within +/-30% of GFR.

*p-value <0.05 for comparing bias in the other equations to that in Pediatric CKID Creatinine-Cystatin Equation based on a two-sided t-test. #p-value <0.05 for comparing variance in bias in the other equations to that in Pediatric CKID Creatinine-Cystatin Equation based on a two-sided F-test. $^a$ their Pearson correlation coefficients ($r$) are almost identical to $\rho$. 

10
Table 2. Change in mGFR on Repeat Evaluation vs Change in eGFR Equations on Repeat Evaluation and Number (%) of Participants with High Accuracy of Repeat Change. 

\[(\text{mGFR}_{\text{repeat}} - \text{mGFR}_{\text{baseline}}) - (\text{eGFR}_{\text{repeat}} - \text{eGFR}_{\text{baseline}})\]

<table>
<thead>
<tr>
<th>Equation</th>
<th>Δ mGFR vs Δ Schwartz Cr</th>
<th>Δ mGFR vs Δ Schwartz BUN Cr</th>
<th>Δ mGFR vs Δ Schwartz CyC</th>
<th>Δ mGFR vs Δ Filler CyC</th>
<th>Δ mGFR vs Δ CKiD CyC &amp; Cr</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>103</td>
<td>103</td>
<td>44</td>
<td>44</td>
<td>42</td>
</tr>
<tr>
<td>Difference in the Change in mGFR vs eGFR on Repeat Evaluation (mL/min/1.73m²), Mean (s.d.)*</td>
<td>-13.8 (20.4)</td>
<td>15.3 (18.5)</td>
<td>45.3 (13.8)</td>
<td>10.5 (16.1)</td>
<td>18.0 (13.0)</td>
</tr>
<tr>
<td>Number and % of patients with Intrasubject Δ bias† &lt; 5 mL/min/1.73m²</td>
<td>6 (5.8%)</td>
<td>6 (5.8%)</td>
<td>7 (15.9%)</td>
<td>6 (13.6%)</td>
<td>7 (16.7%)</td>
</tr>
<tr>
<td>Number and % of patients with Intrasubject Δ bias† &lt; 10 mL/min/1.73m²</td>
<td>18 (17.5%)</td>
<td>21 (20.4%)</td>
<td>17 (40.5%)</td>
<td>12 (27.3%)</td>
<td>17 (40.5%)</td>
</tr>
</tbody>
</table>

*Mean (s.d.) 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})
Figure 1: Correlation between five eGFR equations and mGFR (DTPA)