Published in final edited form as:
*Pediatr Blood Cancer*. 2012 October ; 59(4): 668–674. doi:10.1002/pbc.24100.

# Effect of Hydroxyurea Treatment on Renal Function Parameters: Results from the Multi-Center Placebo-Controlled BABY HUG Clinical Trial for Infants with Sickle Cell Anemia

**Ofelia Alvarez, MD**[1], **Scott T. Miller, MD**[2], **Winfred C. Wang, MD**[3], **Zhaoyu Luo, PhD**[4], **M. Beth McCarville, MD**[3], **George J. Schwartz, MD**[5], **Bruce Thompson, PhD**[4], **Thomas Howard, MD**[6], **Rathi V. Iyer, MD**[7], **Sohail R. Rana, MD**[8], **Zora R. Rogers, MD**[9], **Sharada A. Sarnaik, MD**[10], **Courtney D. Thornburg, MD, MS**[11], **Russell E. Ware, MD, PhD**[12], and **the BABY HUG Investigators**

[(1)]University of Miami, Miami, FL

[(2)]SUNY – Downstate/Kings County Hospital Center Brooklyn, NY

[(3)]St. Jude Children's Research Hospital, Memphis, TN

[(4)]Clinical Trials & Surveys, Corp., Owings Mills, MD

[(5)]University of Rochester Medical Center, Rochester, NY

[(6)]University of Alabama, Birmingham, AL

[(7)]University of Mississippi Medical Center, Jackson, MS

[(8)]Howard University, Washington DC

[(9)]UT Southwestern Medical Center, Dallas, TX

[(10)]Children's Hospital of Michigan, Detroit, MI

[(11)]Duke University Medical Center, Durham, NC

[(12)]Baylor College of Medicine, Houston, TX.

## Abstract

**Background—**Children with sickle cell anemia (SCA) often develop hyposthenuria and renal hyperfiltration at an early age, possibly contributing to the glomerular injury and renal insufficiency commonly seen later in life. The Phase III randomized double-blinded Clinical Trial of Hydroxyurea in Infants with SCA (BABY HUG) tested the hypothesis that hydroxyurea can prevent kidney dysfunction by reducing hyperfiltration.

**Procedure—**193 infants with SCA (mean age 13.8 months) received hydroxyurea 20 mg/kg/day or placebo for 24 months. $^{99m}$Tc diethylenetriaminepentaacetic acid (DTPA) clearance, serum creatinine, serum cystatin C, urinalysis, serum and urine osmolality after parent-supervised fluid deprivation, and renal ultrasonography were obtained at baseline and at exit to measure treatment effects on renal function.

**Results—**At exit children treated with hydroxyurea had significantly higher urine osmolality (mean 495 mOsm/kg $H_2O$ compared to 452 in the placebo group, p=0.007) and a larger

**Corresponding Author:** Ofelia Alvarez, MD, Division of Pediatric Hematology, University of Miami, 1611 NW 12th Avenue, ACC West Room 514, Miami, FL 33136; Phone 305-585-5635; Fax 305-325-8387; oalvarez2@med.miami.edu.

**Conflict of Interest:** The authors have no financial or other conflicts of interest to report.

percentage of subjects taking hydroxyurea achieved urine osmolality >500 mOsm/kg $H_2O$. Moreover, children treated with hydroxyurea had smaller renal volumes (p=0.007). DTPA-derived glomerular filtration rate (GFR) was not significantly different between the two treatment groups, but was significantly higher than published norms. GFR estimated by the Chronic Kidney Disease in Children Schwartz formula was the best non-invasive method to estimate GFR in these children, as it was the closest to the DTPA-derived GFR.

**Conclusion—**Treatment with hydroxyurea for 24 months did not influence GFR in young children with SCA. However, hydroxyurea was associated with better urine concentrating ability and less renal enlargement, suggesting some benefit to renal function.

### Keywords

kidney function; glomerular filtration rate; urine osmolality; hydroxyurea

## INTRODUCTION

Individuals with sickle cell anemia (SCA) are prone to develop dysfunction of multiple organs, including the kidneys. Young children with SCA often develop impaired urine concentrating ability leading to dehydration, renal hyperfiltration related to increased plasma volume, and glomerular enlargement [1]. The current model of sickle cell nephropathy has been summarized recently [2, 3]. Sickling within the renal medulla with consequent destruction of vasa recta is central to the onset of renal damage. Ischemia and hypoxia will also promote the release of vasodilating substances such as prostaglandins and nitric oxide, which may increase glomerular filtration rate. This pathology can present at an early age, and may progress to clinically significant nephropathy with the onset of proteinuria due to changes in glomerular filtration permselectivity [4] over time. Eventually chronic renal failure occurs in a significant number of patients [5]. Hydroxyurea, a drug that increases fetal hemoglobin and reduces erythrocyte sickling, might prevent renal dysfunction if started at an early age [6-9].

BABY HUG was an NHLBI/NICHD-sponsored Phase III double-blinded, placebo-controlled randomized clinical trial (ClinicalTrials.gov # NCT00006400) designed to test the hypothesis that hydroxyurea could prevent chronic organ damage in very young children with SCA. In BABY HUG, the co-primary endpoints were splenic function assessed by qualitative $^{99m}$Tc labeled sulfur colloid liver-spleen scan and renal function (glomerular filtration rate, GFR) assessed by quantitative clearance of technetium-99m ($^{99}$Tc)-DTPA. Multiple secondary endpoints were examined, including several measures of renal pathophysiology. We present the detailed results of the renal assessments after 24 months of study treatment with hydroxyurea or placebo.

## METHODS

### Research subjects and study intervention

The study was conducted in 14 centers throughout the United States; study design and recruitment process have been previously described [10]. Each center contributed a median of 12 (range 4-35) subjects to the study. Parents or guardians signed a consent document approved by the local Institutional Review Board at each center. Children with hemoglobin SS or S-$\beta^0$ thalassemia between the ages of 9-18 months who met the inclusion criteria were assigned in a double-blinded fashion to receive either hydroxyurea at 20 mg/kg given daily as an oral suspension or an equivalent volume of placebo for a total of 24 months. The children were closely monitored for drug toxicity. Overall study adherence was excellent [11].

### Blood and urine specimens to assess renal function

At treatment initiation (baseline) [12] and at exit from the study, serum creatinine was measured centrally at the Georgia Health Sciences University (GHSU) by high performance liquid chromatography (HPLC) to 0.01 mg/dL precision. The remaining serum was used to perform cystatin C levels by particle enhanced immune nephelometry (N Latex Cystatin C; Dade Behring, Deerfield, IL) [13]. This assay has a range of 0.23 to 7.25 mg/L and interassay coefficients of variation of 5.05% and 4.87% at mean concentrations of 0.97 and 1.90 mg/L, respectively. Serum cystatin C analysis was performed centrally at St Jude Children's Research Hospital.

Urine osmolality was measured on urine collected after a scheduled water deprivation. Parents were instructed to withhold food and fluids from their infants overnight for a period close to, but not exceeding, 10 hours. Urine collection bags were provided in order to collect the first morning urine specimen. Serum osmolality was also measured when urine samples were brought to the clinic the following day. Urine and serum specimens were assayed centrally at GHSU by the freezing point depression technique using the Model 2430 Automatic Osmometer Precision System [14]. Isosthenuria was defined as urine osmolality within one standard deviation of mean serum osmolality (280-292 mOsm/kg $H_2O$). Urine hyperosmolality (>292 mOsm/kg $H_2O$) and hypoosmolality (<280 mOsm/kg $H_2O$) were urine osmolalities above or below one standard deviation from the mean serum osmolality, respectively. Urinalysis including specific gravity measurement was performed locally.

### Creatinine-based estimation of GFR

Serum creatinine was used to estimate GFR by the original Schwartz formula [15], the updated "bedside" Schwartz formula [16], and the modified or new CKiD Schwartz formula [16], which incorporates BUN, serum cystatin C, and gender into the GFR estimation. The new Schwartz formula was derived from iohexol clearance measured in children enrolled in the Chronic Kidney Disease in Children (CKiD) cohort [17]. This formula yielded 87.7% of estimated GFR within 30% of the iohexol GFR, and 45.6% within 10% of the iohexol GFR [16].

$$\textbf{Original Schwartz GFR}\left(\mathrm{mL/min/1.73m^2}\right)=\text{height in cm}\times 0.55/\text{serum creatinine}$$

$$\textbf{Bedside/updated Schwartz GFR}\left(\mathrm{mL/min/1.73m^2}\right)=\text{height in cm}\times 0.41/\text{serum creatinine}$$

$$\textbf{Modified or New CKiD Schwartz GFR}\left(\mathrm{mL/min/1.73m^2}\right)=$$
$$39.1\times[\text{height (m)}/\text{serum creatinine (mg/dL)}]^{0.516}\times[1.8/\text{serum cystatin C (mg/L)}]^{0.294}\times$$
$$[30/\text{BUN (mg/dL)}]^{0.169}\times[1.099]^{\text{if male}}\times[\text{height (m)}/1.4]^{0.188}$$

### Cystatin C-based estimation of GFR

There are several formulas to estimate GFR from serum cystatin C [18]. We estimated GFR using a formula previously derived from a comparison between serum cystatin C levels and DTPA clearance in 536 children with various renal pathologies covering a wide range of GFR values [19].

$$\text{GFR}=\text{antilog}\{1.92+[1.123\times\log(1/\text{serum cystatin C})]\}$$

### $^{99m}$Tc-DTPA clearance GFR measurement

$^{99m}$Tc diethylenetriaminepentaacetic acid ($^{99m}$Tc-DTPA) clearance (DTPA-GFR) was used to measure GFR [20, 21]. It was performed at local sites at baseline in all infants and for the majority of subjects at the study exit. Data were analyzed centrally via a computer program

at the Data and Statistical Coordinating Center [12]. Briefly, a single dose of 0.05 μCi/kg (minimum 500 microCuries) of $^{99m}$Tc-DTPA was administered intravenously and plasma samples were collected at 1, 2 and 4 hours after injection according to standard methods, as reported previously [12]. After these specimens were analyzed in duplicate, a plasma DTPA clearance curve was calculated and the quantitative GFR value was derived using the slope-intercept method [22, 23]. This result was then normalized to a body surface area of 1.73 $m^2$.

### Renal ultrasound

Screening sonograms were performed at the participating institutions using a standard scanning procedure developed for BABY HUG [24], and were reviewed centrally. Kidneys were imaged in both supine and prone positions and the maximum longitudinal (L), anterior-posterior (AP) and transverse (T) dimensions were obtained. Renal volumes were calculated in milliliters (mL), using the formula for a prolate ellipsoid: [L x AP x T] x 0.523 as previously described [25]. Kidney length was reported in centimeters. Kidneys were considered abnormally echogenic if the renal cortex was equal to or more echogenic than adjacent spleen or liver. Renal corticomedullary differentiation was considered abnormal when it was difficult to distinguish cortex from medulla visually [26]. Kidneys were also assessed for renal calculi, cysts or other focal parenchymal abnormalities, including infarcts.

### Statistical Analyses

Means and standard deviations were calculated for each parameter used to evaluate renal function. Some laboratory values were $\log_{10}$ transformed to better approximate the normal distribution before data analysis. Clinical characteristics and results of laboratory assessments were compared between hydroxyurea and placebo treatment groups. The comparisons were performed using one way ANOVA and ANCOVA with adjustment of baseline values on compared continuous variables. Comparisons of categorical variables were conducted using either Pearson Chi-square or Fisher's exact test. The Cochran-Armitage test was performed to check the DTPA-GFR trend. Mixed models were utilized to check whether laboratory values were different between DTPA-GFR ≤135 and DTPA-GFR >135 mL/min/1.73$m^2$ in the hydroxyurea and placebo groups. In order to determine if DTPA-GFR at exit was different according to age at treatment initiation in the hydroxyurea group, mixed model regression was also performed. $P<0.05$ was considered statistically significant. All analyses used SAS Version 8.2 & 9.2 (SAS Institute, Cary, North Carolina).

## RESULTS

Table I presents the baseline demographic, laboratory, and radiological data and Table II presents the exit data, separated according to treatment assignment. Table III compares differences between entry and exit by treatment group in those children who had paired entry and exit values. The study population consisted of 193 infants with SCA with a mean age of 13.8 months at the start of the study (range 9-18 months); 179 patients (93%) completed at least 18 months of study treatment and at least one exit assessment, and 167 patients (86%) completed two years of study treatment.

Baseline information has been presented previously [12, 14]. Infants had normal renal function as assessed by serum creatinine and serum cystatin C. Twenty-two percent of the children at baseline had hyposthenuria. Of those who concentrated urine, only 38% (N=55 of 144) had urine osmolality >500 mOsm/kg $H_2O$, which is considered meaningful urine concentration. Only 5% of the children were hyposthenuric at exit.

After 24 months of study intervention with hydroxyurea or placebo, we found significant differences related to treatment. Children who received hydroxyurea had higher urine osmolality after water deprivation than children who received placebo (p=0.007), indicating a better urine concentrating capacity. Forty-two percent (71/168) of the children had urine osmolality >500 mOsm/kg $H_2O$; the percent of children with urine osmolality >500 was higher in the hydroxyurea-treated group (p=0.03). The mean hemoglobin F of children with urine osmolality >500 mOsm/kg $H_2O$ was 5% higher (95% CI 1.83 - 7.2, p-value=0.001) than that of children with urine osmolality ≤ 500. Similarly, children who were taking placebo had lower mean urine specific gravity over time, indicating worsened concentrating ability (p=0.03).

Successful $^{99m}$Tc-DTPA clearance studies were obtained in 176 infants at entry and 142 at exit; after an interim analysis, exit DTPA-GFR was halted by the DSMB because of the statistical conclusion that no difference would be found between the two study groups even if all remaining examinations were performed. At exit, there was no difference in mean GFR between the treatment groups. Mean GFR measured by DTPA clearance was elevated: 146±44 mL /min/1.73m$^2$ in the hydroxyurea arm (74-382) and 146±48mL /min/1.73m$^2$ (18-330) in the placebo arm. The proportions of DTPA-GFR values >135 mL/min/1.73 m$^2$ (90$^{th}$ percentile for normal subjects at age 3 years) within the hydroxyurea and the placebo groups were not different. Age at treatment initiation, total hemoglobin, and hemoglobin F did not impact DTPA-GFR clearance at exit. Figure 1 shows the baseline and exit values for DTPA-GFR in the hydroxyurea treatment arm superimposed on reference curves for infants who received placebo.

We compared the different GFR estimates to DTPA-GFR with the purpose of finding a suitable GFR formula for future use. The correlation coefficients between all DTPA-GFR clearance values and the original Schwartz, the bedside Schwartz formulas, and the CKiD Schwartz estimates were 0.305, 0.305, and 0.299, respectively.

At exit, ultrasound examinations of subjects who received hydroxyurea showed smaller kidney volumes (less hypertrophy) than did ultrasounds of those who received placebo (p =0.007). A minority of children (4%) had sonographic findings of “medical renal disease” either at baseline or at exit (except one child who had persistent findings) with increased renal echogenicity or loss of cortico-medullary differentiation; these findings were not affected by hydroxyurea. None of the children had renal infarcts.

## DISCUSSION

The primary goal of the BABY HUG Clinical Trial was to determine the effect of hydroxyurea in preventing organ dysfunction of the spleen and kidney in infants with SCA. Overall, hydroxyurea was well tolerated. Children assigned to hydroxyurea had documentation of therapeutic benefit with fewer pain and acute chest episodes, and had hematological response with increased fetal hemoglobin, total hemoglobin, and MCV, when compared to placebo [24]. This report discusses the effect of hydroxyurea compared to placebo on renal function parameters; the effect of hydroxyurea on splenic function, the other co-endpoint, will be the focus of another report.

There have been no previous studies comprehensively documenting the various parameters of renal function in young children with SCA. In assessing renal function, several tests were performed to evaluate urine concentrating ability (tubular function) and GFR (glomerular function), in addition to examining anatomic changes by renal sonography. Therefore, this study extends the findings of previously published baseline and overall results of BABY

HUG data to provide valuable new information on renal function for this very young group of children with SCA [12, 27].

At baseline, these children had some measures of renal function that were comparable to those found in normal children. At a mean age of 13 months, the mean serum creatinine was 0.23 mg/dL and serum cystatin 0.9 mg/L, values that are similar to published age norms [25-28]. DTPA-GFR levels showed hyperfiltration (125 mL/min/1.73 $m^2$) compared with the age norm for 51Cr-EDTA-GFR of 91.5 ± 17.8 mL/min/1.73 $m^2$ at 1 year of age [32], and these results are statistically different (p-value <0.001). Although the norms reported in the literature are for $^{51}$Cr-EDTA-GFR, the correlation between $^{99m}$Tc-DTPA and $^{51}$Cr-EDTA plasma clearances is excellent. DTPA may give slightly higher values than EDTA (<5% difference), but this difference is not clinically significant [21, 33].

At exit, the mean DTPA-GFR clearance was 146 mL/min/ 1.73 $m^2$ at age 3 years despite treatment with hydroxyurea [27]. After 2 years of age, normal children have a mean $^{51}$Cr-EDTA-GFR value of 104 ± 19.9 mL/min/1.73 $m^2$ (81 and 135 ml/min/1.73 $m^2$ were the 10$^{th}$ and 90$^{th}$ percentiles, respectively) [32]. Since there are different ways of calculating GFR from DTPA, we decided to reanalyze the data after adjusting the results for the unmeasured fast exponential which may occur within the first 1 hour after DTPA intravenous injection by using the Bröchner-Mortensen quadratic formula (GFR=1.0019 x C1-0.001258 x $C1^2$) [34]. The adjusted values were lower both at entry (110.08±26.32 and 107.81±22.19 in the hydroxyurea and placebo arms, respectively), and at exit (117.49±23.24 and 116.62±29.99 mL/min/1.73 $m^2$ in the hydroxyurea and placebo arms, respectively). However, no treatment effect was observed on GFR (p=0.83). Although lower, the adjusted values were still significantly higher than the reported norms (p=<0.0001 for the hydroxyurea group and 0.0002 for the placebo group).

We analyzed other methods besides DTPA to estimate GFR, including serum cystatin C and cystatin C GFR estimates. Cystatin C is a cysteine protease inhibitor that is produced by all human nucleated cells, and is almost completely filtered by the kidney; therefore, serum levels correlate well with GFR [35]. Serum cystatin C levels were obtained in BABY HUG with the goal of establishing reference values in infants with SCA. The mean serum cystatin C was slightly higher (0.9 mg/L) than expected at age 3, although still within the expected range (norm for children over age 12 months is 0.72±0.12 mg/L (range 0.51-0.95)) [28]. Alvarez et al found a mean serum cystatin C of 0.78 mg/L in an older population of children with SCA (youngest child 9 years of age) without albuminuria [36]. Our study population is the first group of young children with SCA to have serum cystatin C reported. Other factors such as inflammation might have contributed to a slight elevation in the production of cystatin C, which could affect GFR estimation [37].

In this study, other methods that estimated GFR were used and correlated to DTPA-GFR. It is important to underscore that GFR estimates derived from serum creatinine may have been affected by two considerations: (1) The Schwartz formula, bedside Schwartz formula, and CKiD Schwartz formula (derived from plasma iohexol clearance) were developed mainly from patients with poor kidney function, whereas our cohort generally had normal or elevated GFR, and (2) Serum creatinine overestimates GFR due to its secretion by the renal tubules and is affected by low muscle mass [38]. Nevertheless, the Schwartz formula, bedside Schwartz formula and CKiD Schwartz had similar correlation coefficients with the DTPA-GFR (0.305, 0.305, and 0.299 respectively). As the correlations were quite similar, using a formula to estimate GFR could be a matter of preference.

The new CKiD formula takes into consideration two measures of kidney function (serum creatinine and serum cystatin C). The mean value of the difference with the DTPA-GFR was

22.83 (95% CI 17.4 – 36.2, p-value=0.001). This difference was only 4.36 (95% CI 0.19-8.53, p-value=0.041) if the comparison was made with the adjusted DTPA-GFR by the Bröchner-Mortensen correction formula. Thus, we favor the CKiD Schwartz formula as a reasonable non-invasive tool to assess GFR in our patient population without the use of a more time-consuming and invasive method like DTPA clearance. We do not think that it is likely that the use of this formula will result in spuriously low GFRs; obtaining both serum creatinine and serum cystatin may strengthen the evaluation of renal function of these patients.

This study found significant differences in the renal size between the two treatment groups. At 3 years of age the expected normal kidney length is 7.36±0.64 cm [39], which is similar to our reported mean length. Renal volumes correlate to body weight [25]. The expected $50^{th}$ percentile for body weight of a three-year-old boy is 15 kg, which will correspond to an expected combined renal volume of 80 mL. Hydroxyurea therapy impacted renal volume, with treated children having smaller (closer to normal size) kidneys at the end of the study. Differences were not explained by body weight differences between the treatment groups. Children who were on hydroxyurea had a mean weight of 14.5 kg at exit and those on placebo had a mean weight of 14.3 kg [27].

There are few studies reporting norms for urine concentrating ability in children. We observed an improvement over time in urine concentration for the whole group, which it may be related to physiologic maturation in the concentrating capacity, or perhaps the parents were more cautious about water-depriving their children when they were older. A study by Marild [40] described osmolarity determinations testing after a stringent overnight fluid deprivation and intranasal desmopressin; that study found a mean urine osmolality of 825 mOsm/kg $H_2O$ at age 3. Although our fluid deprivation was much less rigorous, the BABY HUG data suggest hydroxyurea treatment preserved urine concentrating ability as evidenced by significant differences in mean urine osmolality and specific gravity and in the proportion of children achieving a urine osmolality >500 mOsm/kg $H_2O$ between treatment groups.

Our study has several limitations. We did not have simultaneous normal controls; comparisons were made with the established norms published in the literature. Another possible limitation is that inulin clearance, which is the gold standard for GFR calculation and consists of both urinary and plasma samples, was not performed; inulin is not available in the United States. However, urine sampling would have required unacceptable added burden of catheterization of our young patients. Furthermore, DTPA clearance correlated very well with inulin clearance in a study done in adults [41]. Another technical problem was the rare infiltration of DTPA radionuclide (estimated in about 3% of the studies), which may have accounted for the few GFRs <81; these were not judged to be clinically relevant because the results did not correlate with other parameters of renal function. Furthermore, these low values did not influence the overall results, as confirmed by a sensitivity analysis that compared the mean values of the coefficient obtained through the regression model for GFR calculation with and without the suspected outliers.

Although BABY HUG did not meet the primary end-point of finding a difference in GFR between treatment groups, it provided useful new information about renal function in this group of patients and demonstrated some benefit on tubular function. A longer follow-up period will be required in order to assess whether hydroxyurea intervention will continue to preserve urine concentrating ability, possibly reducing enuresis, a symptom which is particularly common among children with SCA [42]. Since the natural history of GFR shows a trend for hyperfiltration with increasing age during childhood [43] but eventually patients may develop significant sickle cell nephropathy and renal failure, it will be

important to evaluate the influence of a more extended administration of hydroxyurea on GFR, on the prevention of albuminuria [44], and on the eventual development of renal insufficiency. An observational follow-up study of this cohort of children is ongoing (NCT008903) through 2017 in order to evaluate the long-term effect of hydroxyurea.

## Acknowledgments

We thank the following BABY HUG personnel at the participating Clinical Centers: Children's National Medical Center (Catherine Driscoll, MD; Caterina Minniti, MD; Lori Luchtman-Jones, MD; Brenda Martin MSN, CPNP; Barbara Speller-Brown, MSN), Duke University Medical Center (Sherri Zimmerman, MD; William Schultz, MHS, PA-C; Steven Shipes; Tracy Kelly, MSN, CPNP; Shelly Burgett, FNP), Howard University College of Medicine (Caroline K. Reed, MSN), Johns Hopkins University School of Medicine (James Casella, MD; Phillip Seaman, PA-C; Jeffrey Keefer, MD, PhD; Sue Dixon; Melvin J. Reinhardt; Robert Dejong), Medical University of South Carolina (Ram Kalpatthi, MD; Sherron Jackson, MD; Betsy Rackoff, RN; Lisa Kuisel; Deborah Disco, RNP), St. Jude Children's Research Hospital (Lane Faughnan, BSN; Lynn Wynn, MSN, PNP), SUNY Downstate Medical Center/ Kings County Hospital Center (Kathy Rey, PA-C; Sreedhar Rao, MD; Rebecca S. Gonzalez, CNMT; Arnold M. Strashun, MD), University of Miami (Stuart Toledano, MD; Julio Barredo, MD; Tally Hustace, ARNP), University of Mississippi Medical Center (Glenda Thomas, RN; Deborah Estess, RN; Freida McKenzie, MT; Mary Gail Smith, MD; Tom Hogan, RDMS), University of Texas Southwestern Medical Center at Dallas (Cindy Cochran, MSN, CPNP; Jennifer Marshall, RN; Leah Adix, CCRP), University of Alabama at Birmingham (Jennifer McDuffie, CRNP; Roy McDonald, MPH; Kim Whelan, MD), Drexel University (Lori Luck, MD; Mary Lou MacDermott, CRNP), Emory University School of Medicine (R. Clark Brown, MD, PhD; Ifeyinwa Osunkwo, MD, MPH; Anne Herry; Eldrida Randall; Korin Cherry), and Children's Hospital of Michigan (Wanda Whitten-Shurney, MD; Mary Murphy, MSN, CNP; Rana Khatib, MD). A full listing of contributors to BABY HUG is available at http://www.c-tasc.com/sites/default/files/StudySites/babyhug.html.

We appreciate the valuable contributions of Dr. Amy Piepsz who provided information about the EDTA-GFR norms, and Xuan Huang and Ming Lu who provided statistical assistance.

Finally, we would like to give special thanks to the patients and families of the BABY HUG trial for their participation and commitment.

The BABY HUG trial is supported by the National Heart, Lung and Blood Institute/National Institutes of Health Contracts N01-HB-07150 to N01-HB-07160 with partial support of the Best Pharmaceuticals for Children Act and the National Institute of Child Health and Human Development.

## Abbreviations

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| **$^{99}$Tc-DTPA** | technetium-99m diethylenetriaminepentaacetic acid |
| **GFR** | glomerular filtration rate |
| **SCA** | sickle cell anemia |

## REFERENCES

1. Scheinman JI. Sickle cell disease and the kidney. Nat Clin Pract Nephrol. Feb; 2009 5(2):78–88. [PubMed: 19048000]
2. Becker AM. Sickle cell nephropathy: challenging the conventional wisdom. Pediatr Nephrol. 2011; 26:2099–2109. [PubMed: 21203778]
3. Sharpe CC, Thein SL. Sickle cell nephropathy—a practical approach. Br J Haematol. 2011; 155:287–297. [PubMed: 21902687]
4. Yee, M McPherson; Jabbar, SF.; Osunkwo, I., et al. Chronic kidney disease and albuminuria in children with sickle cell disease. Clin J Am Soc Nephrol. 2011; 6:2628–2633. [PubMed: 21940843]
5. Wong WY, Elliott-Mills D, Powars D. Renal failure in sickle cell anemia. Hematol Oncol Clin North Am. Dec; 1996 10(6):1321–1331. [PubMed: 8956019]
6. Jayabose S, Tugal O, Sandoval C, et al. Clinical and hematological effects of hydroxyurea in children with sickle cell anemia. J Pediatr. 1996; 129(4):559–565. [PubMed: 8859263]

7. Kinney TR, Helms RW, O'Branski EE, et al. Safety of hydroxyurea in children with sickle cell anemia: results of the HUG-KIDS study, a phase I/II trial. Pediatric Hydroxyurea Group. Blood. 1999; 94(5):1550–1554. [PubMed: 10477679]
8. Wang WC, Wynn LW, Rogers ZR, et al. A two-year pilot trial of hydroxyurea in very young children with sickle-cell anemia. J Pediatr. 2001; 139(6):790–796. [PubMed: 11743503]
9. Thornburg CD, Nixon N, Burgett S, et al. A pilot study of hydroxyurea to prevent chronic organ damage in young children with sickle cell anemia. Pediatr Blood Cancer. 2008; 52(5):609–615. [PubMed: 19061213]
10. Thompson BW, Miller ST, Rogers ZR, et al. The pediatric hydroxyurea phase III clinical trial (BABY HUG): challenges of study design. Pediatr Blood Cancer. Feb; 2010 54(2):250–255. [PubMed: 19731330]
11. Thornburg CD, Rogers ZR, Jeng MR, et al. Adherence to study medication and visits: data from the BABY HUG trial. Pediatr Blood Cancer. Feb; 2010 54(2):260–264. [PubMed: 19856395]
12. Ware RE, Rees RC, Sarnaik SA, et al. Renal function in infants with sickle cell anemia: baseline data from the BABY HUG trial. J Pediatr. Jan; 2010 156(1):66–70. [PubMed: 19880138]
13. Newman DJ. Cystatin C. Ann Clin Biochem. Mar; 2002 39(Pt 2):89–104. [PubMed: 11928770]
14. Miller ST, Wang WC, Iyer R, et al. Urine concentrating ability in infants with sickle cell disease: baseline data from the phase III trial of hydroxyurea (BABY HUG). Pediatr Blood Cancer. Feb; 2010 54(2):265–268. [PubMed: 19621454]
15. Schwartz GJ, Haycock GB, Edelmann CM Jr, Spitzer A. A simple estimate of glomerular filtration rate in children derived from body length and plasma creatinine. Pediatrics. 1976; 58:259–263. [PubMed: 951142]
16. Schwartz GJ, Muñoz A, Schneider MF, et al. New equations to estimate GFR in children with CKD. J Am Soc Nephrol. Mar; 2009 20(3):629–637. [PubMed: 19158356]
17. Furth SL, Cole SR, Moxey-Mims M, et al. Design and methods of the Chronic Kidney Disease in Children (CKiD) prospective cohort study. Clin J Am Soc Nephrol. 2006; 1:1006–1015. [PubMed: 17699320]
18. Zappitelli M, Parvex P, Joseph L, et al. Derivation and validation of cystatin C-based prediction equations for GFR in children. Am J Kidney Dis. 2006; 48:221–230. [PubMed: 16860187]
19. Filler G, Lepage N. Should the Schwartz formula for estimation of GFR be replaced by cystatin C formula? Pediatr Nephrol. 2003; 18:981–985. [PubMed: 12920638]
20. Fleming JS, Zivanovic MA, Blake GM, et al. Guidelines for the measurement of glomerular filtration rate using plasma sampling. Nuclear Med Comm. 2004; 25:759–769.
21. Piepsz A, Colarinha P, Gordon I, et al. Guidelines for glomerular filtration rate determination in children. Eur J Nucl Med. 2001; 28(3):BP31–BP36. [PubMed: 11315614]
22. Rehling M, Moller ML, Thamdrup B, et al. Stimultaneous measurement of renal clearance and plasma clearance of $^{99}$mTc-labelled diethylenetrianminepenta-acetate, $^{51}$Cr-labelled ethylenediaminetetra-acetate and inulin in man. Clin Sci. 1984; 66:613–619. [PubMed: 6423339]
23. Rodman JH, Maneval DC, Magill HL, Sunderland M. Measurement of $Tc^{99}$m DTPA serum clearance for estimating glomerular filtration rate in children with cancer. Pharmacotherapy. 1993; 13:10–16. [PubMed: 8437963]
24. Mc Carville MB, Luo Z, Huang X, et al. Abdominal ultrasound with scintigraphic and clinical correlates in very young children with sickle cell anemia: Baseline data from BABY HUG Trial. AJR Am J Roentgenol. Jun; 2011 196(6):1399–1404. [PubMed: 21606305]
25. Dinkel E, Ertel M, Dittrich M, et al. Kidney size in childhood. Sonographical growth charts for kidney length and volume. Pediatr Radiol. 1985; 15:38–43. [PubMed: 3881724]
26. Walker TM, Serjeant GR. Increased renal reflectivity in sickle cell disease: prevalence and characteristics. Clin Radiol. 1995; 50:566–569. [PubMed: 7656526]
27. Wang W, Ware RE, Miller ST, et al. Hydroxycarbamide in very young children with sickle cell-anaemia: a multicentre, randomised, controlled trial (BABY HUG). Lancet. May 14; 2011 377(9778):1663–1672. [PubMed: 21571150]
28. Randers E, Krue S, Erlandsen EJ, et al. Reference interval for serum cystatin C in children. Clin Chem. 1999; 45(10):1856–1858. [PubMed: 10508134]

29. Boer DP, de Rijke YB, Hop WC, et al. Reference values for serum creatinine in children younger than 1 year of age. Pediatr Nephrol. Oct; 2010 25(10):2107–2113. [PubMed: 20505955]
30. Finney H, Newman DJ, Thakkar H, et al. Reference ranges for plasma cystatin C and creatinine measurements in premature infants, neonates, and older children. Arch Dis Child. 2000; 82(1):71–75. [PubMed: 10630919]
31. Fischback M, Graff V, Terzic J, et al. Impact of age on reference values for serum concentration of cystatin C in children. Pediatr Nephrol. 2002; 17(2):104–106. [PubMed: 11875672]
32. Piepsz A, Tondeur M, Ham H. Revisiting normal $^{51}$Cr-ethylenediaminetetraacetic acid clearance values in children. Eur J Nucl Med Mol Imaging. 2006; 33(12):1477–1482. [PubMed: 16865393]
33. Gutte H, Møller ML, Pfeifer AK, et al. Estimating GFR in children with 99mTc-DTPA renography: a comparison with single-sample $^{51}$Cr-EDTA clearance. Clin Physiol Func Imaging. May; 2010 30(3):169–174.
34. Bröchner-Mortensen J, Haahr J, Christoffersen J. A simplified method for accurate assessment of glomerular filtration rate in children. Scand J Clin Lab Invest. 1974; 33:133–143.
35. Bokenkamp A, Domanetzki M, Zinck R, et al. Cystatin C—a new marker of glomerular filtration rate in children independent of height and age. Pediatrics. 1998; 101:875–881. [PubMed: 9565418]
36. Alvarez O, Zilleruelo G, Wright D, et al. Serum cystatin C levels in children with sickle cell disease. Pediatr Nephrol. 2006; 21:533–537. [PubMed: 16491413]
37. Knight EL, Verhave JC, Spiegelman D, et al. Factors influencing serum cystatin C levels other than renal function and the impact on renal function measurement. Kidney Int. 2004; 65:1416–1421. [PubMed: 15086483]
38. Ataga KI, Orringer EP. Renal abnormalities in sickle cell disease. Am J Hematol. 2000; 63:205–211. [PubMed: 10706765]
39. Rosenbaum DM, Korngold E, Teele R Littlewood. Sonographic assessment of renal length in normal children. AJR. 1984; 142:467–469. [PubMed: 6607625]
40. Marild S, Jodal U, Jonasson G, et al. Reference values for renal concentrating capacity in children by the desmopressin test. Pediatr Nephrol. May; 1992 6(3):254–257. [PubMed: 1616834]
41. Fotopoulos A, Bokharhli JA, Tsiouris S, et al. Comparison of six radionuclidic and non-radionuclidic methods for the assessment of glomerular filtration rate in patients with chronic renal failure. Hell J Nucl Med. May-Aug;2006 9(2):133–140. [PubMed: 16894423]
42. Field JJ, Austin PF, An P, et al. Enuresis is a common and persistent problem among children and young adults with sickle cell anemia. Urology. Jul; 2008 72(1):81–84. [PubMed: 18384865]
43. Aygun B, Mortier N, Smeltzer MP, et al. Glomerular hyperfiltration and microalbuminuria in children with sickle cell anemia. Pediatr Nephrol. 2011; 26(8):11285–1290.
44. Alvarez O, Montane B, Lopez G, et al. Early blood transfusions protect against microalbuminuria in children with sickle cell disease. Pediatr Blood Cancer. Jul; 2006 47(1):71–76. [PubMed: 16261557]

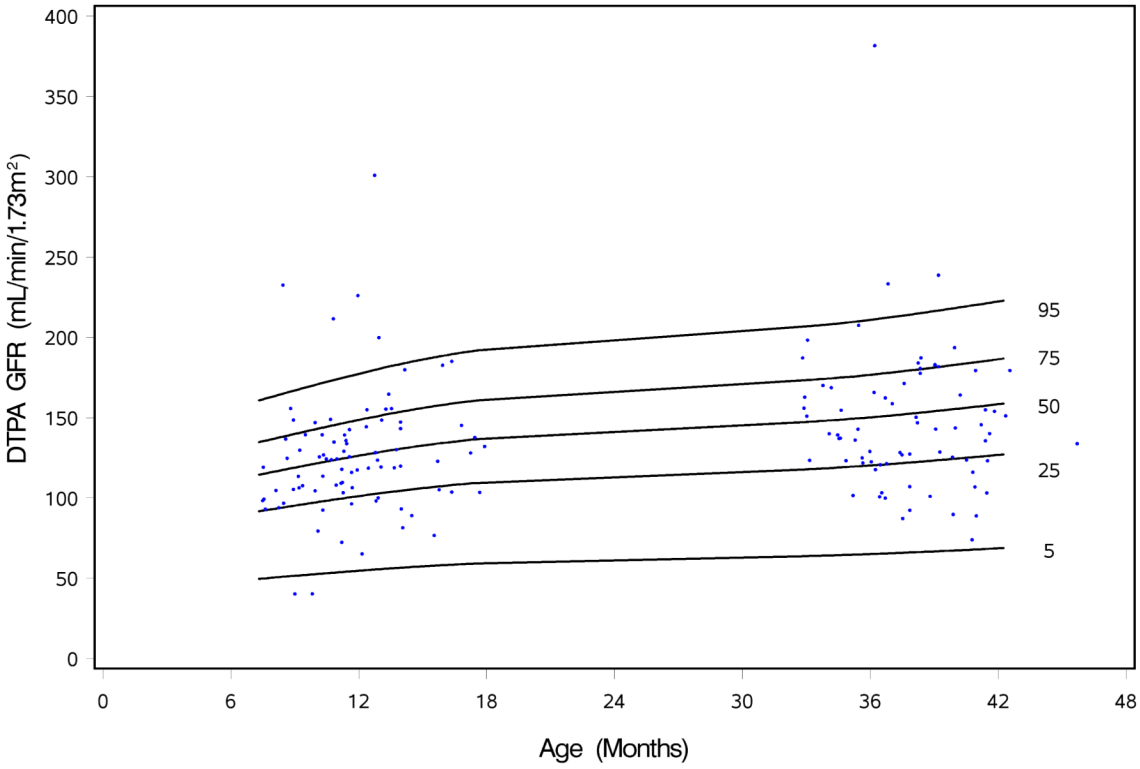


**Figure 1.**
Scatter Plot of Baseline and Exit DTPA-GFR Results in the Hydroxyurea Arm by Age with Placebo Percentile Reference Curves.

**Table I**

Baseline Characteristics and Evaluations of Renal Function

| Parameter | | Hydroxyurea | | Placebo | *P* value* |
|---|---|---|---|---|---|
| | N | % or [Mean/SD] | N | % or [Mean/SD] | |
| Gender | | | | | 0.56 |
| Male | 44 | 46% | 40 | 41% | |
| Female | 52 | 54% | 57 | 59% | |
| Sickle cell diagnosis | | | | | 0.68 |
| Hb SS | 94 | 98% | 93 | 96% | |
| Hb Sbeta$^0$ thal | 2 | 2% | 4 | 4% | |
| Hematology Labs | | | | | |
| Hemoglobin (g/dl) | 96 | 9.00/1.30 | 97 | 9.16/1.32 | 0.41 |
| Hemoglobin F (%) | 94 | 25.93/8.56 | 97 | 25.97/8.45 | 0.97 |
| Log $_{10}$ WBC | 96 | 1.13/0.16 | 97 | 1.12/0.16 | 0.82 |
| Log $_{10}$ reticulocytes | 96 | 2.42/0.22 | 97 | 2.44/0.21 | 0.54 |
| Age (month) | | | | | |
| at start of treatment | 95 | 13.78/2.71 | 96 | 13.77/2.75 | 0.96 |
| Serum creatinine (mg/dL) | 83 | 0.25/0.09 | 90 | 0.23/0.07 | 0.07 |
| Serum cystatin C (mg/L) | 64 | 0.91/0.17 | 71 | 0.90/0.14 | 0.59 |
| Cystatin derived GFR | 64 | 96.41/23.50 | 71 | 96.46/17.02 | 0.99 |
| Original Schwartz GFR | 82 | 190.01/61.19 | 89 | 197.64/48.58 | 0.37 |
| Bedside Schwartz GFR | 82 | 141.65/45.61 | 89 | 141.33/36.21 | 0.37 |
| New CKiD Schwartz GFR | 60 | 99.10/21.22 | 69 | 105.09/17.56 | 0.08 |
| DTPA-GFR (mL/min/1.73 m$^2$) | 86 | 126.42/38.87 | 90 | 124.08/29.76 | 0.65 |
| Urine pH | 96 | 6.47/0.98 | 97 | 6.57/0.98 | 0.47 |
| Urine specific gravity | 96 | 1.011/0.005 | 97 | 1.012/0.006 | 0.09 |
| Serum osmolality mOsm/kg $H_2O$) | 93 | 286.13/5.56 | 94 | 286.45/5.89 | 0.70 |

| Parameter | Hydroxyurea | | Placebo | | *P* value* |
|---|---|---|---|---|---|
| | N | % or [Mean/SD] | N | % or [Mean/SD] | |
| Urine osmolality (mOsm/kg $H_2O$) | 94 | 403.22/151.63 | 97 | 408.32/152.40 | 0.82 |
| Urine osmolality categories | | | | | |
| <280 | 21 | 22% | 20 | 21% | |
| 280-292 | 2 | 2% | 4 | 4% | |
| 293-500 | 42 | 45% | 47 | 48% | 0.91 |
| 501-600 | 22 | 23% | 19 | 20% | |
| >600 | 7 | 8% | 7 | 7% | |
| U osmolality> S osmolality | 70 | 77% | 73 | 78% | 1.0 |
| U osmolality ≤ S osmolality | 21 | 23% | 21 | 22% | |
| Ultrasound, renal volume(mL) | 94 | 61.07/15.05 | 93 | 59.22/15.39 | 0.41 |
| Left kidney | 94 | 31.74/8.45 | 93 | 29.95/8.88 | 0.16 |
| Right kidney | 95 | 29.42/8.21 | 94 | 29.21/8.28 | 0.86 |
| Ultrasound, renal length (cm) | | | | | |
| Left kidney | 95 | 6.28/0.63 | 93 | 6.15/0.65 | 0.19 |
| Right kidney | 95 | 5.99/0.60 | 94 | 5.93/0.66 | 0.48 |

*P-value from F tests comparing continuous variables or from Chi square/Fisher exact tests comparing categorical variables between treatment groups.

**Table II**

Exit Endpoint Results

| Parameter | | Hydroxyurea | | Placebo | *P*-value |
|---|---|---|---|---|---|
| | N | % or [Mean/SD] | N | % or [Mean/SD] | |
| Age (month) at exit | 94 | 37.60/4.30 | 97 | 36.41/5.96 | 0.13 * |
| Length of intervention (month) | 94 | 24.04/3.20 | 97 | 22.87/5.46 | 0. 07 * |
| Hematology Labs | | | | | |
| Hemoglobin | 79 | 9.21/1.31 | 82 | 8.50/1.43 | **<0.001** # |
| Hemoglobin F | 81 | 22.49/8.66 | 79 | 16.99/7.93 | **<0.001** # |
| Log $_{10}$ WBC | 79 | 1.00/0.19 | 82 | 1.12/0.18 | **<0.001** # |
| Log $_{10}$ reticulocytes | 78 | 2.36/0.23 | 81 | 2.45/0.0.27 | **0.004** # |
| Serum creatinine (mg/dL) | 79 | 0.27/0.07 | 84 | 0.25/0.07 | 0.38# |
| Serum cystatin C (mg/L) | 57 | 0.92/0.13 | 59 | 0.90/0.16 | 0.36# |
| Cystatin derived GFR | 57 | 93.75/14.66 | 59 | 96.34/17.00 | 0.12# |
| Original Schwartz GFR | 79 | 215.88/65.89 | 80 | 233.55/90.27 | 0.45# |
| Bedside Schwartz GFR | 79 | 160.93/49.12 | 80 | 174.10/67.29 | 0.45# |
| New CKiD Schwartz GFR | 53 | 112.46/23.10 | 57 | 120.44/26.74 | 0.39# |
| DTPA-GFR (mL/min/1.73m$^2$) | 74 | 146.64/43.70 | 68 | 146.06/48.23 | 0. 93# |
| DTPA-GFR | | | | | 0. 86 * |
| ≤135 | 31 | 42% | 30 | 44% | |
| >135 | 43 | 58% | 38 | 56% | |
| Urine pH | 86 | 6.33/0.87 | 83 | 6.27/0.97 | 0.56# |
| Urine specific gravity | 86 | 1.012/0.004 | 83 | 1.011/0.004 | **0.03** # |
| Serum osmolality | 73 | 283.42/5.17 | 73 | 285.15/10.53 | 0.17# |

| Parameter | | Hydroxyurea | | Placebo | *P*-value |
|---|---|---|---|---|---|
| | N | % or [Mean/SD] | N | % or [Mean/SD] | |
| Urine osmolality | 83 | 494.57/110.07 | 85 | 452.34/92.31 | **0.007** # |
| Categories | | | | | **0.03** * |
| <280 | 4 | 5% | 4 | 5% | |
| 280-292 | 0 | 0% | 1 | 1% | |
| 293-500 | 37 | 45% | 51 | 60% | |
| 501-600 | 27 | 32% | 25 | 29% | |
| >600 | 15 | 18% | 4 | 5% | |
| | | | | | 0.68 * |
| U osmolality> S osmolality | 67 | 94% | 69 | 97% | |
| U osmolality ≤ S osmolality | 4 | 6% | 2 | 3% | |
| Ultrasound, renal volume (mL) | 81 | 91.83/24.41 | 78 | 97.65/21.27 | **0.007** # |
| Left kidney | 81 | 47.68/14.08 | 78 | 48.07/11.07 | 0.26# |
| Right kidney | 83 | 44.15/12.60 | 80 | 50.15/12.75 | **<0.001** # |
| Ultrasound, renal length (cm) | | | | | |
| Left kidney | 82 | 7.36/0.80 | 80 | 7.40/0.64 | **0.03** # |
| Right kidney | 83 | 7.12/0.74 | 80 | 7.18/0.66 | 0.12# |

*p-value from F tests comparing continuous variables or from Chi square/Fisher exact tests comparing categorical variables between treatment groups;

#p-value after adjusted for baseline effect using ANCOVA. The results presented in this table are the results of all tests at study exit. All exit results were obtained after more than 18 months of study intervention. Some of these results may differ slightly from the results in the overall trial report (Wang et al, reference 27) because the data presented in the latter were only for paired results.

**Table III**

Changes in Scores Between Exit and Baseline Data*

| Parameter | N | Hydroxyurea Mean/SD | N | Placebo Mean/SD | *P*-value |
|---|---|---|---|---|---|
| Serum creatinine (mg/dL) | 69 | 0.019/0.11 | 78 | 0.024/0.09 | 0.77 |
| Serum cystatin C (mg/L) | 39 | 0.01/0.19 | 48 | − 0.02/0.19 | 0.42 |
| Urine pH | 86 | − 0.13/1.15 | 82 | − 0.28/1.23 | 0.43 |
| Urine specific gravity | 86 | 0.001/0.005 | 82 | −0.001/0.006 | **0.02** |
| Serum osmolality | 71 | − 3.06/7.26 | 70 | −1.20/9.64 | 0.20 |
| Urine osmolality | 81 | 106.12/179.54 | 84 | 53.71/170.33 | **0.04** |
| Cystatin derived GFR | 39 | − 5.09/25.73 | 48 | 4.00/21.15 | 0.07 |
| Original Schwartz GFR | 69 | 25.47/77.49 | 73 | 33.27/96.7 | 0.60 |
| Bedside Schwartz GFR | 69 | 19.00/57.77 | 73 | 24.70/71.60 | 0.60 |
| New CKiD Schwartz GFR | 33 | 8.85/21.06 | 46 | 14.08/25.12 | 0.30 |
| DTPA-GFR (mL/min/1.73 $m^2$) | 67 | 22.54/54.58 | 66 | 20.73/51.02 | 0.84 |
| Ultrasound, renal volume (mL) | 80 | 29.57/23.01 | 79 | 38.78/22.16 | **0.01** |
| Ultrasound, renal length (cm) | | | | | |
| Length, left kidney | 81 | 1.04/0.58 | 79 | 1.28/0.61 | **0.01** |
| Length, right kidney | 82 | 1.09/0.59 | 79 | 1.28/0.67 | 0.07 |

*Values show difference between mean exit and mean baseline level for patients with paired results. P-value from F tests.