Neonatal Kidney Size and Function in Preterm Infants: What Is a True Estimate of Glomerular Filtration Rate?

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Objectives To distinguish between cystatin C (CysC) and creatinine (Cr) as markers of estimated glomerular filtration rate (eGFR) in preterm infants and to correlate eGFR with total kidney volume (TKV) as a surrogate of nephron mass.

Study design Sixty preterm (<37 weeks' gestational age [GA]) and 40 term infants were enrolled at birth. Serum Cr and CysC levels were assessed during the first week of life. Renal ultrasounds were performed to assess kidney dimensions with calculation of the TKV as a surrogate of nephron mass. Six equations derived from reference inulin, iohexol, and iothalamate clearance studies were used to calculate eGFR. Multiple regression analysis was applied to assess the relative impact of neonatal measures on eGFR, including TKV, GA, and mean arterial pressure (MAP).

Results Renal lengths correlated with GA and were within the reference values for intrauterine measurements. Estimation equations for glomerular filtration rate (GFR) based on Cr, CysC, and combined CysC + Cr demonstrated that Cr-based equations consistently underestimated GFR, whereas CysC and combined equations were more consistent with referenced inulin clearance studies. Term infants demonstrated significantly better eGFR than preterm infants. TKV, GA, and MAP correlated positively with eGFR, although only MAP and GA remained significant when adjusted for other covariates.

Conclusions Primary determinants of eGFR in preterm infants are GA and MAP. The CysC level is a superior biomarker to serum Cr in the assessment of GFR in premature infants. *(J Pediatr 2014;164:1026-31)*.

Preterm infants present important challenges regarding the assessment of neonatal kidney function and potential long-term implications for adult disease.[1-3] Nephron mass is decreased in preterm infants because nephrogenesis is active until 36 weeks' gestation and interruption of gestation results in a loss of total nephron number.[4-6] Moreover, preterm infants are more vulnerable to acute kidney injury (AKI) with the potential loss of nephrons after birth.[7-9] Preterm infants account for more than 80% of AKI in infancy, and protocols for early recognition and follow-up have not been forthcoming.[9] Traditionally, definitions of acute kidney dysfunction are determined solely by serum creatinine (Cr), a derivative of muscle mass, which is very low in preterm infants.[10,11] Also, previous studies indicate placental transfer of maternal Cr and tubular reabsorption in preterm infants, which makes it an inaccurate measure of kidney function in early infancy.[11,12] Alternatively, cystatin C (CysC), a low-molecular-weight protein, is independent of muscle mass and is filtered by the glomerulus and totally metabolized by the renal tubule. It has been proposed as a more reliable marker of glomerular filtration rate (GFR) in infants, although clinical studies are lacking to confirm this.[13,14] Importantly, the "gold standard" of measuring GFR with inulin[14-21] has been replaced by iothalamate[22] or iohexol,[23-25] which has resulted in the development of equations that estimate GFR with the use of Cr or CysC alone or in combination.[22-25]

In this study, we collected simultaneous data on the renal functional markers, CysC and Cr, as well as renal mass in the first week of life in preterm and term infants. The objective was to establish baseline data in infants with minimal perinatal stress. Moreover, the utility of Cr vs CysC was evaluated in an effort to establish reference measures for future studies to define AKI in preterm infants.

AKI	Acute kidney injury
Cr	Creatinine
CysC	Cystatin C
eGFR	Estimated glomerular filtration rate
GA	Gestational age
GFR	Glomerular filtration rate
iGFR	Inulin glomerular filtration rate
MAP	Mean arterial pressure
SES	Socioeconomic status
TKV	Total kidney volume

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Methods

A cross-sectional cohort of 60 preterm (≤37 weeks' gestation) and 40 term (>37 weeks' gestation) singleton infants were enrolled at birth for evaluation of kidney size and function. Demographic and maternal data were recorded, including maternal education, marital status, patient race and ethnicity, and measures of socioeconomic status (SES), determined by zone postal code (ie, ZIP code) from the 2010 census. Gestational age (GA) was determined by fetal ultrasound and postnatal examination as well as projected date of delivery by last menstrual period. Small-for-gestational age was established by weight for GA. Ponderal index (g/cm^3) = [weight (grams) × 100/length^3 (cm^3)] was calculated and applied to the assessment of ponderal size at birth with "lean" being <2 and "heavy" being >2. Infants with known congenital anomalies, including those of the heart, genital-urinary, or gastrointestinal tract, were excluded, as were those with neonatal infections, including those exposed perinatally to HIV. Preterm infants ≤24 weeks' gestation and ≤800 g also were excluded. Nephrotoxic drug use, including aminoglycosides and indomethacin, were monitored. The study was approved by the University of Miami Institutional Review Board, and patient and family privacy was assured under conditions of the Health Insurance Portability and Accountability Act.

Serum Cr was measured by the enzymatic method and was drawn during the first week of life but after the first 48 hours to avoid potential contamination from the maternal Cr. Serum CysC levels were assessed by the central laboratory at the same time as the serum Cr with a particle-enhanced immunonephelometric immunoassay (Dade-Behring, Deerfield, Illinois).

Renal ultrasounds were performed by the pediatric nephrologists using a portable model 8000 4D diagnostic ultrasound system (Apogee Electronics Corp, Santa Monica, California) with a 7.5-MHz small parts probe. Readings were within 3.65% consistency between the 2 performers. The length, width, and anteroposterior diameter (depth) were measured. Kidney volume was calculated in cubic centimeters with the equation for an ellipsoid: volume = length × width × depth × 0.523.[26] Left and right kidney volumes were added for the total kidney volume (TKV; cm^3).

Statistical Analyses

All data sets were tested for normality with the D'Agostino and Pearson omnibus normality test. Intergroup comparisons were tested with ANOVA. Post-test comparisons for significance were performed by the Kruskal-Wallis test for nonparametric data and by the Bonferroni method for parametric data as appropriate. Differences between 2 groups were analyzed by the Student *t* test. Proportional differences were tested with the Fisher exact test.

A review of the literature yielded 7 studies of 302 preterm and term infants with measurements of GFR by inulin clearance (**Table I**; available at www.jpeds.com).[15-21] These data were collated, and a subset of 107 measurements were obtained after 24 hours of birth and within the first week of life and were used to perform Bland-Altman plots for bias and 95% limits of agreement for each of the estimating equations. All GFR data points were matched according to GA.

Univariate correlations were performed with Pearson correlation coefficient (r). Multivariate linear regression analysis was used to test the predictive independent variables on a single constant variable estimated GFR (eGFR). GraphPad Prism (GraphPad Software, Inc, La Jolla, California) and PASW Statistics GradPack 18 (SPSS Inc, Chicago, Illinois) were the statistical programs used to perform the statistical analyses and to construct the graphs. A $P < .05$ was considered significant.

Results

Baseline demographic data that compare term and preterm infants are shown in **Table II**. These data include measures of SES, race/ethnicity, maternal age and parity, Apgar scores, size for GA, ponderal index, and administration of nephrotoxic drugs during the first week of life. It should be noted that our patient population is primarily from an urban and lower SES environment and is heavily weighted towards minority ethnic populations. However, among preterm infants, there were significantly more in the upper affluent SES compared with control patients, reflecting referral patterns to our center for high-risk pregnancies.

Kidney Size

All kidney measurements were closely correlated to GA and to body length ($P < .0001$). The graphs of the regression relationships between right and left kidney length and TKV in neonatal infants relative to GA are shown in **Figure 1** (available at www.jpeds.com).

Table II. Patient demographics

	Term, n = 40	Preterm, n = 60	*P* value
GA, wk	39 ± 1	34 ± 3	<.01
Male sex, n (%)	16 (40)	32 (53)	.72
Race/ethnicity, n (%)			
White	3 (7)	8 (13)	.65
Hispanic	17 (43)	24 (40)	.84
Black	19 (48)	28 (47)	1.00
Asian	1 (2)	0 (0)	.40
SES by zip code, n (%)			
Low: families >20% below poverty	28 (70)	35 (58)	.29
Medium: families >10% to <20% below poverty	9 (23)	11 (18)	.62
High: families <10% below poverty	1 (3)	13 (22)	<.01
Unknown	2 (5)	1 (2)	.56
Apgar score ≤6 at 5 minutes	0 (0)	2 (3)	.51
SGA/IUGR/lean by ponderal index	4 (10)	6 (10)	1.00
Nephrotoxic drugs (first week of life)	4 (10)	26 (43)	<.01
Maternal age, y	29 ± 7	29 ± 8	1.00
Maternal marital status: single, n (%)	28 (70)	47 (78)	.36
Maternal education > high school level, n (%)	8 (21)	12 (20)	1.00

IUGR, intrauterine growth restriction; *SGA*, small-for-gestational age.