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Impact of Gestational Age, Sex, and Postnatal Age on Urine Biomarkers in Premature Neonates

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Abstract

Background—Urine proteins may help understand physiology and diagnose disease in premature infants. Understanding how urine proteins vary by degree of prematurity, sex and postnatal day is warranted.

Methods—We performed a prospective cohort study to assess the independent correlation of 14 urine biomarkers (measured on postnatal days 1–4) with gestational age (GA), sex, and postnatal age in 81 premature infants (mean 1017 grams) without acute kidney injury using random effects mixed model.

Results—Neutrophil gelatinase associated lipocalin (NGAL), and vascular endothelial growth factor (VEGF) showed significant associations for sex, GA, and postnatal age. Cystatin C, Osteopontin (OPN), and Trefoil factor 3 (TFF3) were associated with postnatal age and GA, but not sex. Epithelial growth factor (EGF) and uromodulin were associated with GA only. clusterin was associated with postnatal age and sex. Albumin was associated with sex only. Beta-2-microglobulin (B2M), osteoactivin, kidney injury molecule -1 (KIM-1), and alpha Glutathione S-Transferase (α GST) were associated with postnatal age only.

Conclusion—Postnatal age affects B2M, Cystatin C, NGAL, OPN, Clusterin, Kim-1, Osteoactivin, TFF3, VEGF, α GST. GA affects Cystatin C, EGF, NGAL, OPN, UMOD, TFF3, and VEGF. Sex affects albumin, NGAL, clusterin. Interpretation of urine biomarker will need to account for these associations.

Keywords

Acute kidney injury; acute renal failure; infant; reference; baseline; NGAL; KIM-1; Cystatin C

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INTRODUCTION

Evaluation of urine protein can provide insights into the pathophysiology and diagnosis of disease. We and others have recently shown that urine biomarkers can not only predict acute kidney injury (AKI), but also mortality in premature and near term/term infants.(1, 2) Recently, numerous studies on urine AKI biomarkers, including neutrophil gelatinase-associated lipocalin (NGAL), Cystatin C, and kidney injury molecule-1 (KIM-1) and many others have shown promise in the ability to detect AKI in many critically ill populations. (3–6). These and other markers can also predict hard clinical outcomes such as mortality and could serve to improve our understanding of important pathobiological pathways. AKI is associated with reduced survival and poor outcomes in neonatal and pediatric populations (7–9); yet its diagnosis is challenging as current traditional biomarkers such as serum creatinine (SCr), blood urea nitrogen (BUN), or urine output determine changes in renal function (not damage), therefore, are later indicators of kidney insult and are not specific for renal damage (8, 10, 11). Interpretations of SCr changes in neonates are further complicated as the initial SCr reflects maternal SCr, which improves over the first weeks of life, and inversely related to the degree of immaturity.(12)

For any biomarker, the parameters, which impact serum or urine concentrations, are important to understand before applying their use to predict clinical outcomes. In premature infants, we and others have shown that values for urine biomarkers significantly vary by gestational age (GA)(12). This is likely due to abnormalities in tubular function present in premature infants. Similarly, sex differences affect NGAL levels in premature infants(12, 13) and other cohorts(14). Insight about how urine biomarker concentrations change in the first few days of life, across gestational age categories and sex are critical to make proper inferences about the pathophysiology, the diagnosis of AKI and other outcomes.

In order to study the variability and association between 14 urine biomarkers, with gestational age, sex, and postnatal age, we performed a prospective cohort study on 81 premature infants without AKI. We hypothesize that different urine biomarkers have decreasing/increasing trends over time after birth, differ by GA and sex. To answer our hypotheses, we assessed the independent correlation of 14 biomarkers with GA, sex, and age, after controlling for these variables and their interactions.

STUDY POPULATION AND METHODS

Study population

Very low birth weight (VLBW) infants with birthweight (BW) 1200 grams and/or 31 weeks gestational age admitted to the regional NICU at the University of Alabama at Birmingham (UAB) between February 2012 and June 2013 were enrolled in this prospective cohort study. Newborns were excluded if they had known congenital kidney disease, developed AKI in the first two weeks after birth, or if they did not survive beyond the first 48 hours of life. The study was approved by the UAB Institutional Review Board, informed parental consent was obtained, and LBW infants eligible for the study were enrolled. The clinical and research activities being reported are consistent with the Principles of the Declaration of Helsinki.

For each enrolled infant, infant demographics (i.e., birth weight, GA, sex, race, Apgar scores at 1 and 5 minutes), and maternal demographics (i.e., diabetes, hypertension, smoking, prenatal care, and parity) were collected. Race was defined as Black, White, and Other. GA was categorized as <26 weeks, 26–27 weeks, 28–29 weeks, and ≥30 weeks. In order to ascertain whether a child developed AKI within the first 2 weeks of life, we measured SCr at postnatal days 1, 2, 3, 4, and 12–14 on most infants, in addition to any clinically measured values. The mean number of SCr values obtained for each patient during the first 2 weeks of life was 5 (range 2–14). Neonatal AKI was defined according contemporary definition modified for neonates, similar to KDIGO AKI definition by which a SCr rise by ≥0.3 mg/dl from baseline defines AKI. The lowest previous SCr serves as the baseline for each SCr measurement. The reason that each SCr has a unique baseline level is because on postnatal day 1 SCr reflects maternal creatinine which decreases steadily over the first weeks of life (1,2).

Biomarker Analysis

Urine was collected daily during the first 4 days of life using cuddle buns™ (Small Beginnings, Hersperia, CA, USA) diapers placed at the perineum. The moist part of the diaper was cut out and placed in a syringe. Urine was squeezed into a centrifuge tube using the syringe plunger and centrifuged for 10 minutes to separate urine. The supernatant was aliquoted and frozen at –70° C until the evaluation. Urine biomarker analysis was performed on multi-array plates by electrochemiluminescence using the Sector Image 2400 (Meso Scale Discovery (MSD); Gaithersburg, MD). Albumin, Beta-2-Microglobulin (B2M), Cystatin C, epithelial growth factor (EGF), NGAL, osteopontin (OPN), and uromodulin (UMOD) were measured in urine with MSD Human Kidney Injury Panel 5 Kit assay. Alpha Glutathione S-Transferase (αGST), Calbindin, Clusterin, KIM-1, Osteoactivin, Trefoil factor 3 (TFF3), and vascular endothelial growth factor (VEGF) were measured with MSD Human Kidney Injury Panel 3 Kit Assay. Samples in the panel 3 were diluted 10-fold before being added to the plate. Samples in the panel 5 were diluted 500-fold before being added to the plate. Samples were added to plates and prepared as stated in manufacturer protocol and analyzed on SECTOR Imager. All samples were run in duplicate. All analytes detected in the samples were in the range of the standard curve. The inter-run %CV (coefficient of variance) and intra-run %CV, the variability of samples and standards between and in runs, was below 10% in all cases, and below 5% in most sample/standard comparisons.

Statistical Analysis

Raw biomarker concentrations were recorded as pg/ml, and reported as ng/ml after appropriate unit conversions. These values were corrected for urine creatinine (UCr) concentrations to account for the variability in urine dilution. To report biomarker differences by gestational age (unadjusted by day of life), samples from all 4 days were averaged. Shapiro-Wilk test and normal probability plot were used to test for normality of data. Kruskal-Wallis test was used to examine the differences for non-normal data.

When conducting models to describe the impact of postnatal age on biomarker values, we normalized the data using the natural logarithm (ln). A random effects mixed model was used to examine whether the variance and mean of the biomarkers of interest was dependent

on day of life, sex, and gestational age. In the mixed model analysis, unconditional means model was used to calculate the intraclass correlation coefficient (ICC) in order to determine the proportion of biomarker variance (i.e., due to differences in levels by individual). To examine between- and within-person effects, a linear growth model was used to calculate the proportion of variance (POV) explained for both between and within-person variance. In addition, the effects of gender and GA on mean values of biomarkers were estimated adjusting for day of life (DOL) and the other variable (i.e., the full model). In order to understand the potential confounding effects of gender and GA on the variance by which day affects biomarker level, and also to evaluate their predictive impact on biomarker level, we added GA and gender to the linear growth model with DOL and intercept as the random effect. The proportion of variance explained between-person (POV_b) for GA and gender is the proportion of the explainable variation in biomarker level that can be explained by each predictor after being added to the linear growth model; the associated p-value corresponds to the slope of the mean biomarker level by either parameter. A p-value for fixed effects < 0.05 was considered statistically significant. SAS 9.4 software (SAS Institute Inc., Cary, NC, USA) was used to perform all statistical analyses.

RESULTS

The incidence rate of AKI was 30/111 (27%). We report data for only the 81 infants without AKI. Maternal and infant demographics for the 81 neonates without AKI are shown in table 1. Biomarker concentrations (averaged over the first four days of life) were significantly different across gestational age categories for albumin, cystatin c, EGF, NGAL, UMOD, and osteoactivin (Table 2). After adjusting for urine creatinine, Albumin, Cystatin C, NGAL, OPN, TFF3 were significantly different across gestational age categories (Table 3).

The independent associations of biomarkers with sex, GA, and postnatal age (natural log transformed and corrected for urine creatinine) are shown in Table 4. NGAL, and VEGF showed statistically significant associations for Sex, GA, and postnatal age ($p < 0.05$). Cystatin C, OPN, and TFF3 were associated with postnatal age and GA (all $p < 0.05$), but not sex. EGF and UMOD were associated with GA only ($p < 0.05$). Clusternin was associated with postnatal age and sex $p < 0.05$. Albumin was significantly associated with sex only ($p < 0.05$). B2M, Osteoactivin, KIM1, and α GST were associated with postnatal age only ($p < 0.05$). Calbindin was not associated with GA, postnatal age, or sex. It should be noted that there were no significant interactions between GA, sex and day of life (DOL) (data not shown). To illustrate these relationships Figure 1 demonstrates $\ln$ NGAL over the first 4 days of life by GA. Figure 2 shows $\ln$ NGAL over time by sex.

The between- and within-person variance of the biomarker levels differed among biomarkers. Those with an ICC close to 1 suggest high between-person variance, i.e. Cystatin C (ICC=0.68), NGAL (ICC=0.73), and TFF3 (ICC=0.63).

DISCUSSION

This prospective cohort study provides reference values for 14 candidate urine biomarkers of kidney damage in a sample population of premature infants without AKI. We provide

data which suggest that most biomarker evaluated vary by gestational age and postnatal age, and that sex impacts reference values of albumin, NGAL, Clusterin, and VEGF, but not other studied biomarkers. These findings will be essential in future studies of urine biomarkers' ability to predict AKI and other clinical outcomes in premature infants.

We also report normative ranges for 14 candidate urine biomarkers and urine biomarkers (corrected by urine creatinine) in premature infants without AKI. We report the, 2.5, 25, mean 75 and 97.5 percentiles for each biomarker according to gestational age categories. This data will be useful as a reference range for infants without AKI. Importantly, these reference values must be taken in context of several factors. First, the cohort had predefined inclusion criteria and more importantly the way we excluded those infant with AKI accoding the neonatal KDIGO AKI definition modified for newborns, which is an empiric working definition which may change over time. Second, the method of urine collection (for example is one use a urine bag, different types of cotton fibers balls, or directly from an indwelling urine catheter) may alter the values we publish herewith.

Several articles investigate the use of urinary biomarkers as a predictive and diagnostic tool for AKI in different populations (15–20). Lavery et al.(21) reported that NGAL has negative association with birth weight, which could be regarded a proxy for gestational age and in accordance with our results; however they did not find such association with DOL. This is likely due to small sample size in their study (N=20). A study by Huynh et al.(13) investigated urine NGAL measured between days 4–30 of life in premature infants in whom the likelihood of AKI was low, as they only measured urine in VLBW infants with uncomplicated courses. They found relatively lower overall levels of NGAL, which may reflect tubular maturity, and thus less urine NGAL. Our study corroborated their findings that NGAL concentrations are higher in female premature infants, decrease with higher gestational age and higher postnatal age. In a study of 48 premature infants, Genc et al.(19) concluded that there is no association between urinary KIM-1 level and either GA or sex and we can confirm their findings in our study of premature infants. These data corroborated our previous work (22) where we show that NGAL, Cystatin C, KIM-1, OPN, and B2M were inversely correlation with GA, and female sex was associated with higher NGAL. After excluding newborns with AKI in current study, the correlation of GA with KIM-1 and B2M is diminished (data not shown).

The strength of this study includes the comprehensive analysis of 14 potential candidate kidney injury biomarkers, and exclusion of infants who had AKI, using contemporary definitions, and large number of SCr values. We chose to analyze the 14 urine biomarker values as we were aiming to expand our knowledge of urine biomarkers in premature infants using the same technology/procedures we have done previously. Thus, we used the 7 plex mesoscale Acute kidney injury Kit 3. In addition, because we wanted to explore the role of KIM-1 in this cohort, and we wanted to maintain similar procedures, we added Acute Kidney Injury Kit 5. Doing the entire panel that had already been validated by the Mesoscale was the most efficient and effective way to perform the studies. We had adequate sample size to understand the biological pattern of change in the first postnatal days, and the modifying factors of GA, sex, and the interaction of all three variables. Additionally, we measured ICC and POV which indicate how much variability exist in biomarker

concentrations and how much of that variability can be explained by GA, sex, and postnatal age. However, we acknowledge the limitations in our study: not all infants had urine samples collected for all four days, which could potentially decrease the accuracy of predictions. No differences in demographic characteristics were evident by the number of urine samples assessed, and thus we do not have any reason to believe that those individuals with missing values were systematically different to introduce bias. We also acknowledge that not all of the variability can be accounted for by the variables which we explored, as suggested by POVs. Hence, other variables and confounding effects (besides GA, sex and postnatal age) could impact baseline biomarker levels in premature infants. Considering our sample size, we could not include other potential modifying parameters in our study. It is important to bear in mind that these results reflect the characteristics of premature infants without AKI, and a comparative evaluation of these characteristics in AKI patients is essential before any interpretation.

In conclusion, in this patient population, postnatal age affects B2M, Cystatin C, NGAL, OPN, Clusterin, Kim-1, Osteoactivin, TFF3, VEGF, and α GST; Gestational age affects Cystatin C, EGF, NGAL, OPN, UMOD, TFF3, and VEGF; sex impacts albumin, NGAL, clusterin, and VEGF. It is necessary to account for these associations and variations when designing studies to investigate the diagnostic role of urinary biomarkers and clinical outcomes, and when using urine proteins to understand the pathophysiology of disease. Larger cohorts are needed to facilitate the exploration of more parameters that could potentially impact these biomarkers in VLBW infants.

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Figure 1. Mean and distribution (range) for urine neutrophil gelatinase-associated lipocalin (NGAL) corrected for urine creatinine over time by gestational age (GA) in 81 premature infants without acute kidney injury (AKI)

Figure 2.

Mean and distribution (range) for urine neutrophil gelatinase-associated neutrophil gelatinase-associated lipocalin (NGAL) corrected for corrected for urine creatinine over time by gender in 81 premature infants without acute kidney injury (AKI)

Table 1

Demographics among 81 neonates without acute kidney injury (AKI)

Infant Demographics	
Birth Weight (Mean $\pm$ SD)	1017 $\pm$ 298
Apgar 1 min (Median, IQR)	5 (2–6)
Apgar 5 min (Median, IQR)	7 (7–8)
Gender (N, %)	
Female	38 (46.9)
Race (N, %)	
Black	44 (54.3)
White	30 (37.0)
Other	7 (8.7)
Gestational Age (N, %)	
<26 weeks	13 (16.1)
26–27 weeks	20 (24.7)
28–29 weeks	30 (37.0)
30 weeks	18 (22.2)
Maternal Demographics (N, %)	
Prenatal care	76 (93.8)
Diabetes	8 (9.9)
Hypertension	21 (25.9)
Smoking	10 (12.4)
Multiparity	22 (27.2)

Table 2

Average absolute biomarker concentration (during the first 4 days) across gestational age categories

Concentra- tion (ng/ml)	<26 wk (N=13)			26–27 wk (N=20)			28–29 wk (N=30)			30 wk (N= 18)			p [*]
	Me- dian	IQR	2.5 %– 97.5 %	Me- dian	IQR	2.5% – 97.5 %	Me- dian	IQR	2.5%– 97.5 %	Me- dian	IQR	2.5 %– 97.5 %	
Albumin	24200	22300–42273	12614–99564	18866	13396–37453	3216–246705	26714	16536–45713	5578–2408044	17196	11100–21692	6154–72746	<0.04
B2M	8876	7325–12325	1725–23700	10242	6036–12485	1928–19546	8606	4652–11200	515–25465	8561	6172–15244	2769–27900	0.83
CystatinC	1354	493–1807	75–2962	844	320–1173	33–4863	358	157–830	32–9713	209	85–632	18–3702	0.03
EGF	0.75	0.61–1.04	0.51–1.77	0.79	0.53–1.02	0.35–2.85	1.40	1.07–1.62	0.39–5.12	1.45	1.02–1.80	0.39–2.81	<0.001
NGAL	298	194–640	153–3409	276	110–406	37–6943	210	139–559	7–2928	48	33–211	8–2460	0.01
OPN	466	395–638	180–1439	373	261–642	90–2734	410	239–728	59–1651	347	239–517	106–1850	0.59
UMOD	744	613–955	345–2910	977	763–1367	272–2766	1373	1126–2034	279–5193	1447	953–1902	168–7671	0.001
Clusterin	177	115–194	79–468	165	75–217	15–537	176	111–261	25–1384	115	80–315	23–1341	0.51
KIM-1	3.16	1.68–4.53	0.86–9.69	1.91	1.14–2.59	0.39–6.64	2.63	1.41–3.95	0.52–33.15	2.04	0.99–2.92	0.72–7.34	0.23
Osteoactivin	1.25	0.88–1.72	0.45–2.36	1.11	0.88–1.39	0.37–5.73	1.53	1.31–2.42	0.50–8.50	1.58	1.34–2.34	0.61–3.65	0.03
TFF3	9.34	4.80–24.35	1.53–41.84	7.71	4.60–15.42	0.92–72.17	10.81	6.34–16.20	1.08–44.38	5.21	2.83–8.47	0.93–62.41	0.17
VEGF	0.94	0.74–1.94	0.51–3.54	0.75	0.58–1.13	0.31–7.09	1.01	0.79–1.34	0.20–3.12	0.72	0.50–1.35	0.40–5.24	0.15
Calbindin	6.32	3.96–7.31	0.98–13.10	3.79	2.78–7.68	0.97–20.59	5.90	3.67–9.11	1.30–36.88	6.01	4.91–15.26	1.87–35.11	0.20
GST	0.21	0.06–0.53	0.03–1.21	0.13	0.01–0.30	0.00–1.78	0.09	0.04–0.24	0.00–49.60	0.18	0.04–0.92	0.00–86.17	0.48
UCr (mg/dl)	11.9	10.0–13.2	2.3–14.9	8.9	7.1–11.4	4.6–32.6	13.4	10.6–17.7	7.1–33.5	12.4	10.1–18.8	6.8–23.9	0.006

* Estimated from a Kruskal-Wallis test

Table 3

Average biomarker concentration (during the first 4 days) corrected for urine creatinine (UCr) across gestational age categories

	<26 wk (N=13)				26–27 wk (N=20)				28–29 wk (N=30)				30 wk (N=18)				p*
	Medi-an	IQ R	2.5% – 97.5 %	Medi-an	IQ R	2.5% – 97.5 %	Medi-an	IQ R	2.5% – 97.5 %	Medi-an	IQ R	2.5% – 97.5 %	Medi-an	IQ R	2.5% – 97.5 %		
Albumin	2.12×10 ⁻¹	1.74×10 ⁻¹ , 2.94×10 ⁻¹	1.06×10 ⁻¹ , 4.28	2.22×10 ⁻¹	1.43×10 ⁻¹ , 2.90×10 ⁻¹	4.76×10 ⁻² , 2.18	1.81×10 ⁻¹	9.77×10 ⁻² , 3.71×10 ⁻¹	5.77×10 ⁻² , 1.20×10	1.21×10 ⁻¹	8.71×10 ⁻² , 1.68×10 ⁻¹	4.86×10 ⁻² , 6.03×10 ⁻¹	4.86×10 ⁻² , 6.03×10 ⁻¹	8.71×10 ⁻² , 1.68×10 ⁻¹	4.86×10 ⁻² , 6.03×10 ⁻¹	0.04	
B2M	7.38×10 ⁻²	6.08×10 ⁻² , 1.09×10 ⁻¹	1.34×10 ⁻² , 5.09×10 ⁻¹	9.93×10 ⁻²	4.70×10 ⁻² , 1.64×10 ⁻¹	2.07×10 ⁻² , 2.12×10 ⁻¹	7.16×10 ⁻²	3.32×10 ⁻² , 9.13×10 ⁻²	1.54×10 ⁻³ , 1.60×10 ⁻¹	7.71×10 ⁻²	5.26×10 ⁻² , 9.54×10 ⁻²	1.46×10 ⁻² , 2.45×10 ⁻¹	1.46×10 ⁻² , 2.45×10 ⁻¹	5.26×10 ⁻² , 9.54×10 ⁻²	1.46×10 ⁻² , 2.45×10 ⁻¹	0.20	
CystatinC	1.15×10 ⁻²	4.56×10 ⁻³ , 2.07×10 ⁻²	5.81×10 ⁻⁴ , 5.82×10 ⁻²	8.98×10 ⁻³	3.30×10 ⁻³ , 1.26×10 ⁻²	4.85×10 ⁻⁴ , 2.96×10 ⁻²	3.03×10 ⁻³	9.61×10 ⁻⁴ , 7.38×10 ⁻³	2.48×10 ⁻⁴ , 4.24×10 ⁻²	1.50×10 ⁻³	5.25×10 ⁻⁴ , 3.45×10 ⁻³	1.90×10 ⁻⁴ , 4.82×10 ⁻²	1.90×10 ⁻⁴ , 4.82×10 ⁻²	5.25×10 ⁻⁴ , 3.45×10 ⁻³	1.90×10 ⁻⁴ , 4.82×10 ⁻²	0.006	
EGF	7.60×10 ⁻⁶	6.20×10 ⁻⁶ , 9.40×10 ⁻⁶	3.38×10 ⁻⁶ , 2.63×10 ⁻⁵	8.30×10 ⁻⁶	5.90×10 ⁻⁶ , 1.00×10 ⁻⁵	5.08×10 ⁻⁶ , 1.63×10 ⁻⁵	1.02×10 ⁻⁵	7.40×10 ⁻⁶ , 1.37×10 ⁻⁵	4.68×10 ⁻⁶ , 2.14×10 ⁻⁵	1.07×10 ⁻⁵	8.80×10 ⁻⁶ , 1.31×10 ⁻⁵	5.39×10 ⁻⁶ , 1.40×10 ⁻⁵	5.39×10 ⁻⁶ , 1.40×10 ⁻⁵	8.80×10 ⁻⁶ , 1.31×10 ⁻⁵	5.39×10 ⁻⁶ , 1.40×10 ⁻⁵	0.14	
NGAL	2.48×10 ⁻³	1.94×10 ⁻³ , 1.32×10 ⁻²	1.14×10 ⁻³ , 3.22×10 ⁻²	2.67×10 ⁻³	1.19×10 ⁻³ , 5.39×10 ⁻³	4.28×10 ⁻⁴ , 6.13×10 ⁻²	1.84×10 ⁻³	9.66×10 ⁻⁴ , 4.32×10 ⁻³	7.59×10 ⁻⁵ , 1.28×10 ⁻²	4.47×10 ⁻⁴	2.75×10 ⁻⁴ , 1.25×10 ⁻³	5.83×10 ⁻⁵ , 2.04×10 ⁻²	5.83×10 ⁻⁵ , 2.04×10 ⁻²	2.75×10 ⁻⁴ , 1.25×10 ⁻³	5.83×10 ⁻⁵ , 2.04×10 ⁻²	0.002	
OPN	4.88×10 ⁻³	2.71×10 ⁻³ , 5.79×10 ⁻³	1.51×10 ⁻³ , 1.97×10 ⁻²	4.27×10 ⁻³	3.03×10 ⁻³ , 6.80×10 ⁻³	1.06×10 ⁻³ , 2.41×10 ⁻²	2.98×10 ⁻³	2.00×10 ⁻³ , 4.00×10 ⁻³	6.10×10 ⁻⁴ , 1.38×10 ⁻²	2.58×10 ⁻³	1.79×10 ⁻³ , 3.77×10 ⁻³	1.05×10 ⁻³ , 1.58×10 ⁻²	1.05×10 ⁻³ , 1.58×10 ⁻²	1.79×10 ⁻³ , 3.77×10 ⁻³	1.05×10 ⁻³ , 1.58×10 ⁻²	0.02	
UMOD	6.76×10 ⁻³	5.91×10 ⁻³ , 7.55×10 ⁻³	2.69×10 ⁻³ , 2.75×10 ⁻²	1.24×10 ⁻²	7.46×10 ⁻³ , 1.54×10 ⁻²	3.71×10 ⁻³ , 3.42×10 ⁻²	1.09×10 ⁻²	9.26×10 ⁻³ , 1.37×10 ⁻²	2.71×10 ⁻³ , 2.46×10 ⁻²	1.04×10 ⁻²	8.21×10 ⁻³ , 1.29×10 ⁻²	2.48×10 ⁻³ , 3.80×10 ⁻²	2.48×10 ⁻³ , 3.80×10 ⁻²	8.21×10 ⁻³ , 1.29×10 ⁻²	2.48×10 ⁻³ , 3.80×10 ⁻²	0.06	
Clusterin	1.35×10 ⁻³	9.30×10 ⁻⁴ , 2.46×10 ⁻³	5.40×10 ⁻⁴ , 6.85×10 ⁻³	1.37×10 ⁻³	7.11×10 ⁻⁴ , 2.13×10 ⁻³	2.17×10 ⁻⁴ , 4.74×10 ⁻³	1.21×10 ⁻³	7.07×10 ⁻⁴ , 2.23×10 ⁻³	2.14×10 ⁻⁴ , 9.40×10 ⁻³	8.98×10 ⁻⁴	7.63×10 ⁻⁴ , 1.59×10 ⁻³	1.15×10 ⁻⁴ , 1.77×10 ⁻²	1.15×10 ⁻⁴ , 1.77×10 ⁻²	7.63×10 ⁻⁴ , 1.59×10 ⁻³	1.15×10 ⁻⁴ , 1.77×10 ⁻²	0.67	
KIM-1	2.71×10 ⁻⁵	1.87×10 ⁻⁵ , 4.28×10 ⁻⁵	5.76×10 ⁻⁶ , 3.83×10 ⁻⁴	2.03×10 ⁻⁵	1.43×10 ⁻⁵ , 2.92×10 ⁻⁵	4.52×10 ⁻⁶ , 4.00×10 ⁻⁵	1.62×10 ⁻⁵	9.40×10 ⁻⁶ , 3.41×10 ⁻⁵	3.21×10 ⁻⁶ , 2.97×10 ⁻⁴	1.35×10 ⁻⁵	8.50×10 ⁻⁶ , 2.38×10 ⁻⁵	3.62×10 ⁻⁶ , 5.80×10 ⁻⁵	3.62×10 ⁻⁶ , 5.80×10 ⁻⁵	8.50×10 ⁻⁶ , 2.38×10 ⁻⁵	3.62×10 ⁻⁶ , 5.80×10 ⁻⁵	0.14	
Osteoactivin	1.32×10 ⁻⁵	8.50×10 ⁻⁶ , 1.73×10 ⁻⁵	2.98×10 ⁻⁶ , 3.77×10 ⁻⁵	1.25×10 ⁻⁵	9.70×10 ⁻⁶ , 1.58×10 ⁻⁵	4.49×10 ⁻⁶ , 2.44×10 ⁻⁵	1.18×10 ⁻⁵	8.10×10 ⁻⁶ , 1.74×10 ⁻⁵	4.28×10 ⁻⁶ , 4.9×10 ⁻⁵	1.24×10 ⁻⁵	9.40×10 ⁻⁶ , 1.75×10 ⁻⁵	3.21×10 ⁻⁶ , 3.47×10 ⁻⁵	3.21×10 ⁻⁶ , 3.47×10 ⁻⁵	9.40×10 ⁻⁶ , 1.75×10 ⁻⁵	3.21×10 ⁻⁶ , 3.47×10 ⁻⁵	0.99	
TFF3	1.05×10 ⁻⁴	4.44×10 ⁻⁵ , 2.81×10 ⁻⁴	1.14×10 ⁻⁵ , 4.01×10 ⁻⁴	9.48×10 ⁻⁵	5.33×10 ⁻⁵ , 1.42×10 ⁻⁴	1.37×10 ⁻⁵ , 3.16×10 ⁻⁴	7.90×10 ⁻⁵	4.39×10 ⁻⁵ , 1.12×10 ⁻⁴	7.20×10 ⁻⁶ , 3.93×10 ⁻⁴	4.28×10 ⁻⁵	1.82×10 ⁻⁵ , 6.70×10 ⁻⁵	5.60×10 ⁻⁶ , 5.18×10 ⁻⁴	5.60×10 ⁻⁶ , 5.18×10 ⁻⁴	1.82×10 ⁻⁵ , 6.70×10 ⁻⁵	5.60×10 ⁻⁶ , 5.18×10 ⁻⁴	0.04	
VEGF	1.20×10 ⁻⁵	6.90×10 ⁻⁶ , 1.83×10 ⁻⁵	5.03×10 ⁻⁶ , 2.68×10 ⁻⁵	8.20×10 ⁻⁶	6.70×10 ⁻⁶ , 1.23×10 ⁻⁵	3.60×10 ⁻⁶ , 2.18×10 ⁻⁵	7.30×10 ⁻⁶	5.10×10 ⁻⁶ , 1.19×10 ⁻⁵	2.08×10 ⁻⁶ , 2.62×10 ⁻⁵	5.70×10 ⁻⁶	4.70×10 ⁻⁶ , 9.10×10 ⁻⁶	2.10×10 ⁻⁶ , 4.34×10 ⁻⁵	2.10×10 ⁻⁶ , 4.34×10 ⁻⁵	4.70×10 ⁻⁶ , 9.10×10 ⁻⁶	2.10×10 ⁻⁶ , 4.34×10 ⁻⁵	0.07	
Calbindin	5.89×10 ⁻⁵	4.14×10 ⁻⁵ , 6.49×10 ⁻⁵	7.60×10 ⁻⁶ , 1.64×10 ⁻⁴	3.70×10 ⁻⁵	2.93×10 ⁻⁵ , 7.02×10 ⁻⁵	1.44×10 ⁻⁵ , 1.79×10 ⁻⁴	4.47×10 ⁻⁵	2.65×10 ⁻⁵ , 7.25×10 ⁻⁵	7.35×10 ⁻⁶ , 1.81×10 ⁻⁴	5.32×10 ⁻⁵	3.64×10 ⁻⁵ , 1.09×10 ⁻⁴	9.35×10 ⁻⁶ , 2.50×10 ⁻⁴	9.35×10 ⁻⁶ , 2.50×10 ⁻⁴	3.64×10 ⁻⁵ , 1.09×10 ⁻⁴	9.35×10 ⁻⁶ , 2.50×10 ⁻⁴	0.31	
GST	2.10×10 ⁻⁶	5.00×10 ⁻⁷ , 5.70×10 ⁻⁶	2.20×10 ⁻⁷ , 1.15×10 ⁻⁵	1.30×10 ⁻⁶	1.00×10 ⁻⁷ , 3.90×10 ⁻⁶	0, 1.20×10 ⁻⁵	7.00×10 ⁻⁷	3.00×10 ⁻⁷ , 2.00×10 ⁻⁶	0, 4.44×10 ⁻⁴	1.20×10 ⁻⁶	4.00×10 ⁻⁷ , 3.80×10 ⁻⁶	0, 1.27×10 ⁻³	0, 1.27×10 ⁻³	4.00×10 ⁻⁷ , 3.80×10 ⁻⁶	0, 1.27×10 ⁻³	0.43	

* Estimated from a Kruskal-Wallis test

Table 4

Intraclass correlation coefficient (ICC) and proportion of variance (POV) explained by postnatal age (DOL), gestational age, and gender

	Postnatal Age				Gestational Age				Gender	
	ICC*	POV _w [†]	P (unadjusted)	p (Full model)	POV _b [‡]	p (DOL-adjusted)	p (Full model)	POV _b	p (DOL-adjusted)	p (Full model)
ln Albumin/Cr	0.53	0.26	0.67	0.62	-0.61	0.2	0.13	-0.58	0.08	0.05
ln B2M/Cr	0.41	0.41	<0.001	<0.001	-0.38	0.07	0.07	-0.54	0.84	0.71
ln CystatinC/Cr	0.68	0.24	<0.001	<0.001	-0.21	0.005	0.004	-0.49	0.36	0.25
ln EGF/Cr	0.53	0.02	0.31	0.4	-0.16	0.004	0.002	0.03	0.15	0.08
ln NGAL/Cr	0.73	0.22	<0.001	<0.001	-0.18	<0.001	<0.001	-0.46	0.02	0.003
ln OPN/Cr	0.61	0.25	0.009	0.01	-1.04	0.04	0.04	-1.21	0.29	0.26
ln UMOD/Cr	0.35	0.28	0.06	0.07	-2.34	0.001	0.001	-2.32	0.27	0.29
ln Clusterin/Cr	0.59	0.14	<0.001	<0.001	-0.22	0.19	0.13	-0.12	0.02	0.01
ln KIM-1/Cr	0.59	0.24	<0.001	<0.001	-0.66	0.08	0.09	-0.92	0.97	0.95
ln Osteonectin/Cr	0.53	0.12	0.002	0.002	-0.65	0.87	0.88	-0.61	0.89	0.95
ln TFF3/Cr	0.63	0.40	0.001	0.002	-0.63	0.02	0.02	-0.81	0.43	0.28
ln VEGF/Cr	0.51	0.14	<0.001	<0.001	0.13	0.02	0.001	0.27	<0.001	<0.001
ln Calbindin/Cr	0.47	0.35	0.19	0.2	-1.77	0.69	0.7	-1.73	0.71	0.74
ln GST/Cr	0.51	0.44	<0.001	<0.001	-1.35	0.21	0.22	-1.50	0.5	0.54

* ICC: Intraclass Correlation Coefficient

[†] POV_w: Proportion of Variance explained within-person

[‡] POV_b: Proportion of Variance explained between-person