



Published in final edited form as:

*Pediatr Res.* 2022 July ; 92(1): 151–167. doi:10.1038/s41390-021-01814-x.

## Gestational age, sex, and time affect urine biomarker concentrations in extremely low gestational age neonates

David J. Askenazi<sup>1,\*</sup>, Brian A. Halloran<sup>1</sup>, Patrick J. Heagerty<sup>2</sup>, Robert H. Schmicker<sup>2</sup>, Patrick Brophy<sup>3</sup>, Sandra E. Juul<sup>4</sup>, Sangeeta Hingorani<sup>4</sup>, Stuart L. Goldstein<sup>5</sup> PENUT Trial Consortium

<sup>1</sup>Department of Pediatrics, University of Alabama at Birmingham, Birmingham, AL

<sup>2</sup>Department of Biostatistics, University of Washington, Seattle, Washington

<sup>3</sup>Department of Pediatrics, University of Rochester/Golisano Children's Hospital, Rochester, NY

<sup>4</sup>Department of Pediatrics, University of Washington/Seattle Children's Hospital, Seattle, WA

<sup>5</sup>Department of Pediatrics, Cincinnati Children's Hospital Medical Center/University of Cincinnati College of Medicine, Cincinnati, OH

### Abstract

**Background:** Our understanding of the normative concentrations of urine biomarkers in premature neonates is limited.

---

Users may view, print, copy, and download text and data-mine the content in such documents, for the purposes of academic research, subject always to the full Conditions of use:[http://www.nature.com/authors/editorial\\_policies/license.html#terms](http://www.nature.com/authors/editorial_policies/license.html#terms)

\***Corresponding Author:** David J. Askenazi, MD, MsPH, Department of Pediatrics, Division of Nephrology, University of Alabama at Birmingham, [daskenazi@peds.uab.edu](mailto:daskenazi@peds.uab.edu), Phone: +1-205-638-9781, Fax: +1-205-996-7590, Postal address: Lowder 516, 1600 7<sup>TH</sup> Avenue South, Birmingham, AL 35233, USA.

#### Author Contributions

DA contributed to the conceptualization and design of the study, data analysis, and drafted the initial manuscript. BH, RS, PH, PB, SJ, SG, and SH contributed to the conceptualization and design of the study, data analysis and assisted in the manuscript preparation. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

#### Prior Presentations

Some data presented in this study were previously presented as an e-poster abstract presentation at the 2020 American Society of Nephrology meeting.

#### Disclosures

All authors declare no real or perceived conflicts of interest that could affect the study design, collection, analyses, and interpretation of data, writing of the report, or the decision to submit for publication. For full disclosure, we provide here an additional list of other authors' commitments and funding sources that are not directly related to this study:

Dr. Askenazi is a consultant for Baxter, Nuwellis, Medtronic, Bioporto, AKI foundation and SeaStar. He also receives external education and research funding not related to this project from Baxter, Nuwellis, and Medtronic.

Dr. Goldstein reports personal fees from and a position as a consultant to Nuwellis, Renibus, ExThera, Reata and Medtronic Inc. Dr. Goldstein receives grant funding from and serves as a consultant and on a Speaker's Bureau for Baxter Healthcare, Inc. Dr. Goldstein receives grant funding and serves as a consultant for BioPorto, Inc. Dr. Goldstein serves on a Speaker's Bureau for Fresenius Medical Corporation. Dr. Juul receives grant funding from NINDS and NICHD for studies not related to this project. Dr. Heagerty and Mr. Schmicker receive grant funding from NHLBI and PCORI for studies not related to this project.

**Category of Study:** Clinical Research – Original Manuscript

**Consent Statement:** Parental/ Guardian consent was required for participation.

#### Ethics Committee Approval

The University of Washington Institutional Review Board (IRB) approved this collaborative study, and each center received approval from their respective IRBs.

**Methods:** We evaluated urine from 750 extremely low gestational age neonates (ELGANs) without severe acute kidney injury (AKI) to determine how gestational age (GA) affects 10 different urine biomarkers at birth and over the first 30 postnatal days. Then we investigated if the urine biomarkers changed over time at 27, 30, and 34 weeks postmenstrual age (PMA). Next, we evaluated the impact of sex on urine biomarker concentrations at birth and over time. Finally, we evaluated if urine biomarkers were impacted by treatment with erythropoietin (Epo).

**Results:** We found that all 10 biomarker concentrations differ at birth by GA and that some urine biomarker concentrations increase while others decrease over time. At 27 weeks PMA, 7/10 urine biomarkers differed by GA. By 30 weeks PMA, 5/10 differed and by 34 weeks PMA only osteopontin differed by GA. About half of the biomarker concentrations differed by sex, and 4/10 showed different rates of change over time between males vs. females. We found no differences in urine biomarkers by treatment group.

**Conclusions:** The temporal, GA and sex differences need to be considered in urine AKI biomarker analyses.

---

## Introduction

Neonatal acute kidney injury (AKI) is common and negatively impacts survival<sup>1-4</sup> in critically ill neonates; yet, the current diagnostic approach using serum creatinine and urine output is challenging in premature neonates.<sup>5-8</sup> Studies in premature infants suggest that certain urine biomarkers can predict who will develop AKI<sup>9-16</sup>, mortality,<sup>15,17</sup> and chronic lung disease.<sup>18,19</sup> Urine biomarkers may provide a non-invasive way to diagnose kidney disease early in the disease process, clarify the underlying cause of AKI, and identify patients who may most benefit from therapeutic intervention, which can improve the care of neonates at risk for kidney related disease. In addition, urine biomarker concentrations and patterns over time may inform developmental and pathobiological pathways.

A clear understanding of normal urine biomarker concentrations and urine biomarker/creatinine ratios is needed. Specifically, understanding how these biomarkers differ at birth and over time by gestational age (GA) and sex is important. Furthermore, understanding whether these biomarkers ‘normalize’ at different developmental timepoints is needed before we can apply them to clinical practice. Using small single-center cohorts, we and others have shown that urine biomarker concentrations vary by GA in the first postnatal week.<sup>20-23</sup> Sex impacts the normative concentrations of some, but not all, urine biomarker concentrations in premature<sup>8,23</sup> and term neonates.<sup>24</sup> A more detailed evaluation using larger sample sizes over a longer period of time is needed to understand how differences in the degree of prematurity affect urine biomarker concentrations over chronological time and over time from conception. The term postmenstrual age (PMA) is used to define time from conception (GA plus chronological age). The changes in urine biomarkers as a function of PMA in premature neonates have been reported for oxytocin<sup>25</sup> and corpoporphin,<sup>26</sup> but not for urine candidate AKI biomarkers.

We evaluated 10 urine biomarkers and their respective urine biomarker/creatinine ratios from birth to 30 days in 750 extremely low GA neonates (24 - 27<sup>6/7</sup> weeks GA) without severe AKI to understand how the degree of prematurity, sex, chronological time,

developmental time, and erythropoietin (Epo) impact urine biomarker values. We evaluated the following urine biomarkers: albumin, beta-2-microglobulin (B2M), cystatin C, epithelial growth factor (EGF), neutrophil gelatinase-associated lipocalin (NGAL), osteopontin (OPN), and uromodulin (UMOD), Kidney Injury Molecule-1 (KIM-1), clusterin, and alpha-glutathione-S-transferase ( $\alpha$ GST). Our research questions include:

1. Do urine biomarker concentrations, biomarker/creatinine ratios differ by GA at birth? Do the temporal patterns differ by GA over the first 30 postnatal days?
2. Do urine biomarker concentrations and urine biomarker/creatinine ratio differ at 27, 30, and 34 weeks PMA by GA?
3. Do urine biomarker concentrations, biomarker/creatinine ratios differ by sex at birth? Do the temporal patterns differ by sex over the first 30 postnatal days?
4. Do urine biomarker concentrations, differ in those randomized to Epo vs. Placebo at birth? Do the temporal patterns differ by Epo Status over the first 30 postnatal days?

## Methods

### Patient Population

The Preterm Erythropoietin Neuroprotection Trial (PENUT) is a randomized, placebo-controlled double-blind clinical trial of recombinant Epo in 24-28 week GA neonates performed across 19 academic centers and comprised of 30 NICUs across 13 states in the United States from December 2013 - September 2016. PENUT screened 3366 neonates, of whom 941 were enrolled in the study. Of those who met the inclusion / exclusion criteria, the main reason for exclusion from PENUT included: parents uninterested, research team unavailable, participation in a competing study, and died before screening. The description of randomization, reasons for non-enrollment, the neurocognitive and kidney-related outcomes for this randomized trial have been described in detail elsewhere.<sup>27,28</sup>

Of the 941 subjects enrolled in the study, 750 met the inclusion/ exclusion criteria for the current analysis. The reasons for exclusion are outlined in Figure 1 (4 were not randomized, 1 was ineligible, 13 died within 2 days of birth, 7 did not have urine biomarkers collected, and 166 had at least one episode of severe AKI, defined as a doubling of serum creatinine from baseline according to stage 2 or 3 of the neonatal KDIGO AKI definition at any point in the NICU stay).<sup>4,29</sup> Subjects with severe AKI were excluded aprior as this analysis is designed to evaluate the effect of GA, time, and sex on biomarker levels, and elimination of those with severe AKI is necessary given that severe AKI causes sharp changes in the concentration of urine biomarkers. We chose to keep those with stage 1 AKI as we and others have shown that a small rise in serum creatinine (SCr) of 0.3 mg/dL is extremely common in ELGANs, as they establish a steady-state SCr in the first days after birth,<sup>30,31</sup> and may not represent true kidney damage/injury.

We use the term gestational age (GA) as the number of weeks and days from conception; postnatal age as the time elapsed after birth; and postmenstrual age (PMA) to define time

from conception (GA plus chronological age) as previously described.<sup>32</sup> Day of birth is defined as day 0.

### Urine collection and analysis

We collected urine using a cotton ball in the diaper. As this was an ancillary proposal to an existing study, the collection of urine was not mandatory, although sites were encouraged to get urine at postnatal days 1, 3, 5, 7, 9, 14, 21, 28, and at 30 and 34 weeks PMA. Of the 750 subjects, urine was collected in 679 (day 0-1), 242 (day 2-3), 374 (day 4-5), 484 (day 6-7), 202 (day 8-9), 623 (day 13-15), 540 (day 20-22), and 519 (day 27-29). Urine was available for 689 at 27 week PMA, 492 at 30 week PMA and 325 at 34 week PMA.

Urine was frozen at the individual sites and remained in a  $-70^{\circ}\text{C}$  freezer (and under dry ice during shipment) until analysis. We chose urine biomarkers which have been reported in the literature as plausible biomarkers of AKI.<sup>10,33,34</sup> Urine was analyzed at the University of Alabama at Birmingham on multi-analyte electrochemiluminescent with a Meso QuickPlex SQ120 multiplexing imager (Meso Scale Discovery (MSD), Gaithersburg, MD). Albumin, B2M, cystatin C, EGF, NGAL, OPN, and UMOD were measured with MSD Human Kidney Injury Panel 5. Urine was diluted 500-fold before being added to plates. Urine KIM-1, clusterin, and  $\alpha\text{GST}$  were assayed with an MSD custom 3-plex assay and were diluted 10-fold before being added to plates. Samples were prepared as per manufacturer protocol and analyzed on the multiplexing imager. Samples were run in duplicate, and the average concentration was used for reporting. The coefficient of variation (%CV) for 9 of the 10 biomarker concentrations was excellent with a range from 0.47 to 5.6%. The %CV for B2M was poor at 29.6% (likely because 41% of the samples were higher than the upper level of detection on the standard curve). Urine creatinine was measured using tandem mass spectroscopy (UAB O'Brien Core Center for Acute Kidney Injury Research) (%CV = 3.8%). We assigned a concentration as the lower detection concentration / 2 for results lower than the lowest detection limit. We assigned a concentration as the average of the highest detected concentration and the detection limit for results that were higher than the highest limit of detection.

### Statistical analyses

The cohort is divided into four GA groups (24, 25, 26 and 27 weeks). Baseline demographic data by GA group are shown in Table 1. We rounded down to nearest GA week (e.g. neonates who had a GA of 24 weeks + 0-6 days are categorized as 24 weeks). Continuous demographic variables are compared between GA groups using means and standard deviations. Categorical variables are reported as the total number and percentage.

Biomarker concentrations are reported as pg/mL for consistency, noting that some of the biomarker values will be represented as value times  $1 \times 10^x$  pg/mL. Urine creatinine is reported as mg/dL. The urine biomarker/creatinine ratios (pg/mL divided by mg/dL) are reported as a unitless term multiplied by a coefficient of  $1 \times 10^{-7}$ . When comparing biomarker concentration differences between groups, we transformed the biomarker data to log<sub>10</sub> values to account for skewedness in the data. We transformed to original scale to

present results. Figures are presented in log<sub>10</sub> scale. For each biomarker we performed the following analyses:

- A.** Differences between biomarkers at birth and % changes per week by GA (Table 2 and Figure 2). To evaluate differences in biomarker concentrations among GA groups, we use linear regression models with generalized estimating equations (GEE) to account for potential within sibship correlation.<sup>35</sup> The GEE models include GA, time, GA\*time interaction, and account for mother as a clustering variable. For each biomarker and biomarker/cr ratio, we used the following mean model:  $\beta_0 + \beta_{\text{DAY}} \text{DAY} + \beta_{\text{GA25}} 1(\text{GA}=25) + \beta_{\text{GA26}} 1(\text{GA}=26) + \beta_{\text{GA27}} 1(\text{GA}=27) + \beta_{\text{DAY*GA25}} \text{DAY} * 1(\text{GA}=25) + \beta_{\text{DAY*GA26}} \text{DAY} * 1(\text{GA}=26) + \beta_{\text{DAY*GA27}} \text{DAY} * 1(\text{GA}=27)$ . Table 2 shows based on testing the GA indicators, whether the predicted concentration differ at birth (intercept), and whether the concentrations differ in their rate of change over time (slope) based on testing for group-by-time interactions. Similar report and analysis are performed for urine biomarker/cr ratios. Figure 2 shows the urine biomarker concentration using a 7-day rolling mean (day X +/- 3 days) on a log<sub>10</sub> scale for each of the four GA groups.
- B.** Differences in biomarker concentrations and biomarker / creatinine ratios by PMA. Table 3 shows the means (95% CI) for the 10 biomarkers and creatinine at 27, 30, and 34 weeks PMA for the 4 groups, and the mean (95% CI) for the 10 biomarkers/creatinine ratios. To evaluate differences between biomarkers by GA groups, we again use linear regression models with GEE to account for within sibship correlation. The GEE models include GA at birth, time measured in PMA, and accounts for mother as a clustering variable. For each biomarker we used the following mean model:  $\beta_0 + \beta_{\text{PMA}} \text{PMA} + \beta_{\text{GA25}} 1(\text{GA}=25) + \beta_{\text{GA26}} 1(\text{GA}=26) + \beta_{\text{GA27}} 1(\text{GA}=27) + \beta_{\text{PMA*GA25}} \text{PMA} * 1(\text{GA}=25) + \beta_{\text{PMA*GA26}} \text{PMA} * 1(\text{GA}=26) + \beta_{\text{PMA*GA27}} \text{PMA} * 1(\text{GA}=27)$ . We evaluated for significant differences in the following:
- Differences in biomarker concentration over time anchored to PMA for each of the four GA groups biomarker =  $\beta_0 + \beta_{\text{GA25}} 1(\text{GA}=25) + \beta_{\text{GA26}} 1(\text{GA}=26) + \beta_{\text{GA27}} 1(\text{GA}=27)$  (p-values reported in rows in Table 3) (p-values in rows in Table 3).
  - Differences in the biomarkers by GA group (columns in Table 3) at each of the 3 timepoints (27, 30, and 34 weeks PMA) based on linear model  $\beta_0 + \beta_{\text{PMA}} \text{PMA}$  (p-values reported in columns in Table 3).
- Similar analyses for urine biomarker/cr ratio are presented in Table 3. Figure 3 shows the median (IQR) urine biomarkers on a log<sub>10</sub> scale at 27, 30 and 34 weeks ( $\pm$  3 days) PMA, for each of the 4 groups (24, 25, 26, and 27 weeks GA).
- C.** Differences between biomarkers at birth and % changes per week by GA sex. To evaluate differences in biomarker concentrations over time by sex, we use linear regression models with GEE including sex, time, a sex\*time interaction

and accounting for mother as a clustering variable. Predicted values for each biomarker were obtained for each GA at birth (time=0). Table 4 shows the means and CI for the urine biomarker concentrations at birth (intercept) and reports differences in the concentrations over time (slope) by sex testing the sex-by-time interaction. Similar analyses are shown in Table 4 for urine biomarker/cr ratios. Figure 4 shows mean urine biomarkers concentrations using a 7-day rolling mean (day X +/-3 days) on a log10 scale by sex.

- D. Differences between biomarkers at birth and % changes per week by randomized group (Placebo vs Epo).** To evaluate differences in biomarker concentrations by treatment group, we use linear regression models with GEE including group, time, a group\*time interaction and accounting for mother as a clustering variable. Predicted values for each biomarker were obtained for the Epo and placebo group at birth (time=0). Table 6 shows the means and CI for the urine biomarker concentrations at birth (intercept) and reports differences in the concentrations over time (slope) by group testing the sex-by-time interaction.

Data management and analysis were conducted using R version 5.3.1 (R Foundation for Statistical Computing, Vienna, Austria). A p-value < 0.05 was considered statistically significant. The University of Washington Institutional Review Board (IRB) approved this collaborative study, and each center received approval from their respective IRBs.

## Results

750 neonates met criteria for study inclusion; 163, 188, 187, and 212 were categorized in the 24, 25, 26, and 27-week GA groups, respectively. Approximately half (N = 380) were male, and half (N = 370) were female. Demographic characteristics for the 750 subjects by GA groups are outlined in Table 1a. Demographic characteristics for the 750 subjects by sex are outlined in Table 1b.

Tables 2 - 5 and Figures 2 - 4 provide information about each of the stated hypotheses for each biomarker and each biomarker/cr ratio. We provide a summary of the analysis for each of the biomarkers in Table 6.

As no statistical differences in biomarker values were found between those randomized to Epo vs. Placebo, we do not provide these in the summary paragraphs below and instead refer the reader to Table 5. The correlation between biomarker concentrations are shown in figure 5.

We summarize the key findings for the hypotheses one biomarker and biomarker/cr ratios at a time, presented in alphabetical order

### **$\alpha$ GST**

Figure 2 shows the log10 mean urine  $\alpha$ GST concentration over the first 30 postnatal days. Table 2 how that at birth, the mean  $\alpha$ GST in those born at 24 weeks GA = 25.9 (20.4 ,33.0). The value at birth sequentially decreased to a mean of 8.64 (7.07, 10.6) among neonates born at 27 weeks GA. In addition, the % change over time decreased with increasing GA



(rate of change for the 24, 25, 26 week GA groups, respectively. The % change over time increased in those who were 27 weeks GA at birth ( $p<0.001$ ). similar findings were seen for  $\alpha$ GST/cr ratios.

Next, report of the urine  $\alpha$ GST concentrations,  $\alpha$ GST /Cr ratios and an assessment of whether these values converged at the 27, 30 and 34 week PMA timepoints was performed. Table 3 and Figure 3 show the  $\alpha$ GST concentrations and  $\alpha$ GST/Cr ratios were not statistically significantly different at 27, 30 and 34 weeks timepoints.

Finally, Figure 4 and Table 4 show that while mean (sd)  $\alpha$ GST at birth was lower in males than females, (13.5 (11.6, 15.8) vs. 19.8 (16.8, 23.3);  $p<0.001$ ), the rate of decline did not differ over the first 30 days between males and females. Similar findings were seen for mean  $\alpha$ GST/cr ratio.

### Albumin

Figure 2 shows the log10 mean urine albumin concentrations over the first 30 postnatal days by GA. At birth the mean (sd) albumin concentrations were highest in the 24 week GA group which decreased steadily with increasing GA ( $p<0.001$ ). In addition, the rate of change in albumin increased systematically across GA groups with a small and statistically significant increase by increasing GA ( $p<0.001$ ). Similar findings were seen for urine albumin/cr ratio by GA at birth and over time.

Next, report whether the urine albumin and albumin/cr concentrations converged at the 27, 30, and 34-week PMA timepoints. Figure 3 show the log10 mean albumin concentration over time. Table 3 shows that the albumin ( $p<0.001$ ) and albumin/cr differed at the 27-week PMA timepoint, but not the 30- and 34- week timepoints.

Finally, Figure 4 and Table 4 show that albumin and albumin /cr ratios were similar at birth between males and females. However, although the rate of change increased for both males and females, the rate of change was higher for males ( $p<0.05$ ). Similar findings were seen for albumin/cr by sex.

### B2M

Figure 2 show the log10 mean urine B2M concentration over the first 30 postnatal days. At birth the mean B2M was highest in the 24 week GA group which systematically decreased with increasing GA ( $p<0.001$ ). The rate of differed by GA groups ( $p<0.05$ ). Similar findings were seen for B2M/cr ratios.

Next, we report urine B2M concentrations, B2M/Cr ratios at different timepoints and assess whether these values converged at the 27, 30- and 34-week PMA timepoints. Figure 3 and Table 3 show the mean B2M concentration and the B2M/Cr ratios differed by GA at the 27 week PMA timepoints ( $p<0.05$ ), but the levels converge at 30- and 34-weeks PMA.

Finally, Figure 4 and Table 4 show that B2M was lower in males than females ( $p<0.05$ ). Urine B2M concentration increased slightly over the first 30 days in males , while it

decreased slightly per week in females ( $p<0.05$ ). Similar findings were seen for B2M /cr ratio by sex.

### Clusterin

Figure 2 show the mean log<sub>10</sub> urine clusterin concentrations over the first 30 postnatal days. Table 2 shows that at birth the mean clusterin values was highest in the 24 week GA group and decreased systematically with increasing GA ( $p<0.001$ ) The clusterin values were not statistically different over time. Similar findings were noted for clusterin/cr ratios.

Next, we report urine clusterin, clusterin /Cr ratios at the 27, 30- and 34-week PMA timepoints. Figure 3 and Table 3 show that the mean clusterin concentrations ratios differed at the 27-week ( $p<0.001$ ) and 30-week PMA ( $p<0.05$ ) by GA group, but the levels converge to similar concentrations at 34 weeks PMA. Similar findings were noted for clusterin/cr ratios.

Finally, Figure 4 and Table 4 show that at birth, clusterin was slightly lower in males than females ( $p<0.05$ ). Urine clusterin increased slightly over the first 30 days in both males and females (not statistically different). Similar findings were seen for Clustein /cr ratio by sex

### Cystatin C

Figure 2 show the mean log<sub>10</sub> urine cystatin c concentrations over the first 30 postnatal days. At birth the mean cystatin values were highest in the 24 weeks GA and were systematically lower with increasing GA ( $p<0.001$ ). The cystatin C percent change per week decreased slightly in the 24 week GA group, but increased systematically across the other GA groups ( $p<0.001$ ). Similar findings were seen for cystatin C/cr ratio evaluations.

Next, we report urine cystatin c and cystatin c / Cr ratios at the 27, 30 and 34 week PMA timepoints. Figure 3 and Table 3 show the mean cystatin C concentrations at the 27 week PMA timepoint differed by GA group ( $p<0.05$ ), but the levels had achieved similar concentrations at 30 and 34 weeks PMA for each of the GA groups. When evaluating the cystatin C/Cr ratios, there were statistically significant differences at the 24 week ( $p<0.001$ ) and 27 weeks ( $p<0.05$ ) PMA timepoints, but not at 30 week PMA.

Finally, Figure 4 and Table 4 show that cystatin C was lower in males than females ( $p<0.001$ ). Urine cystatin C increased in males with a rate of rise per week while there was not a significant change in females ( $p<0.05$ ). Similar findings were seen for cystatin/cr ratio by sex.

### EGF

Figure 2 show the mean log<sub>10</sub> urine EGF concentrations over the first 30 postnatal days. At birth EGF was lowest for the 24 week GA and increased systematically increased by increasing GA ( $p<0.001$ ). The rate of change of urine EGF was not statistically different across GA groups. Similar findings were found for EGF/Cr ratio at birth; however, we found a statistically significant increase in EGF/Cr ratio by increasing GA group over time.



Next, report of the urine EGF concentrations, EGF /Cr ratios converged at the 27, 30- and 34-week PMA timepoints. Figure 3 and Table 3 show the mean EGF concentrations and the EGF/Cr ratios did not differ significantly at the 27 or 34 week PMA timepoints but differed at the 30 week timepoint ( $p<0.05$ ).

Finally, Figure 4 and Table 4 show that EGF was similar in males and females at birth and urine EGF increased over the first 30 days) in both males and females. Alternatively, the EGF/Cr ratio differed by sex at birth; yet the rate of change was not statistically different by sex.

### **KIM-1**

Figure 2 show the mean log<sub>10</sub> urine KIM-1 concentrations over the first 30 postnatal days. Table 2 shows that KIM-1 was highest at birth in the 24 week GA group, which steadily decreased by increasing GA group ( $p<0.001$ ). The rate of change increased systematically across GA groups, although these changes were not statistically significant. Similar findings were seen for KIM-1/cr values.

Next, report of the urine KIM-1 concentrations, KIM-1 /Cr ratios at the 27, 30 and 34 week PMA timepoints was performed. Figure 3 and Table 3 show the average KIM-1 concentration at the 27 and 30 week PMA timepoints differed by GA group (both  $p<0.001$ ), but the concentrations were no longer statistically different at the 34 weeks PMA. When evaluating urine KIM-1/cr ratios, difference were seen at only the 27 week timepoint.

Finally, Figure 4 and Table 4 show that KIM-1 was slightly lower in males vs females ( $p<0.05$ ). Urine KIM-1 increased slightly over the first 30 days for both sexes to a similar degree. Similar findings were seen for mean KIM-1 /cr ratio.

### **NGAL**

Figure 2 show the mean log<sub>10</sub> NGAL concentrations over the first 30 postnatal days. Table 2 shows that at birth NGAL was highest in the 24 week GA group, which decreased steadily with increasing GA ( $p<0.001$ ). The rate of change decreased systematically across GA groups with very similar rates of decline by GA. Similar findings were found for urine NGAL/Cr values.

Next, report of the urine NGAL concentrations, NGAL/Cr ratios and an assessment of whether these values converged at the 27, 30 and 34 week PMA timepoints was performed. Figure 3 and Table 3 show that the mean NGAL concentrations and the NGAL/Cr ratios were statistically different at the 27 week and 30 week PMA timepoints by GA group, but the levels were no longer statistically different at the 34 weeks PMA timepoint.

Finally, Figure 4 and Table 4 show that NGAL concentration at birth was higher in females than in males ( $p<0.001$ ). The urine NGAL for both sexes decreased slightly over the first 30 days. Similar findings were seen for mean NGAL /cr ratio by sex.

## OPN

Figure 2 shows the mean log 10 urine OPN concentrations over the first 30 postnatal days. Table 2 shows that at birth the mean OPN was highest in the 24 week GA and systematically decreased with increasing GA. In addition, the rate of change decreased across GA groups with a statistically significant difference by GA ( $p<0.05$ ). Similar findings were found for the OPN/ Cr values at birth ( $p<0.001$ ); but no statistically significant differences over time were observed for OPN/Cr by GA.

Next, report of the urine OPN and OPN /Cr ratios at the 27, 30 and 34 week PMA timepoints. Figure 3 and Table 3 show the mean OPN concentrations was significantly different at the 30 and 34 week timepoints (but not the 27 week PMA timepoint). The OPN/Cr ratios differed at the 27, 30 and 34 week PMA timepoints.

Finally, Figure 4 and Table 4 show that urine OPN was similar in males and females. Urine OPN for both sexes decreased similarly over the first 30 days. Similar findings were seen for mean OPN /cr ratio by sex.

## UMOD

Figure 2 show the mean log10 urine UMOD concentrations over the first 30 postnatal days. Table 2 shows that at birth UMOD was lowest for the 24 week GA and systematically increased with higher GA ( $p<0.001$ ). The rate of change increased slightly over time by GA groups, although these changes were not statistically significant. Similar findings are observed for the UMOD/Cr ratios.

Next, report of the UMOD and UMOD /Cr ratios at the 27, 30 and 34 week PMA timepoints. Figure 3 and Table 3 show that UMOD concentrations were not statistically different by GA at the 27, 30, and 34-week PMA timepoints. The mean UMOD/cr ratio was significantly different at the 27 weeks PMA timepoint, but did not differ at the 30 and 34 weeks timepoint.

Finally, Figure 4 and Table 4 show that UMOD concentrations are similar in males vs females. The mean log10 change over time increased slightly for males and females over the first 30 days. Similar findings were seen for mean UMOD /cr ratio.

## Creatinine

Figure 2 show the mean log10 urine creatinine concentration over the first 30 postnatal days. Table 2 shows that the 24 week GA had the lowest urine creatinine increased systematically with increasing GA group ( $p<0.001$ ). The rate of change steadily increased slightly across GA groups (differences not statistically different).

Next, report of the urine creatinine concentrations at the 27, 30 and 34 week PMA timepoints was performed. Figure 3 and Table 3 show that the mean urine creatinine concentrations at the 27 week PMA timepoint differed slightly by GA group at the 27 week PMA timepoints, but were not statistically different at the 30 and 34 weeks PMA timepoints.

Finally, Figure 4 and Table 4 show that creatinine at birth was similar in males and urine creatinine increased slightly for both sexes over the first 30 days without significant difference by sex.

## Summary of Results

Table 6 summarizes the findings in Tables 2, 3, 4, and 5. The mean biomarker concentrations at birth for all 10 urine biomarkers differed by GA. The biomarkers which showed an increase in the rate of change over the first 30 days were  $\alpha$ GST, NGAL, and OPN. The biomarkers that showed decrease in the rate of change over the first 30 days were albumin, clusterin, creatinine EGF, KIM-1 and UMOD. The biomarkers that showed different rates of change over time by GA group were  $\alpha$ GST, albumin, B2M, cystatin C, and OPN.

The biomarker concentrations that differ at 27 weeks PMA include albumin, B2M, clusterin, creatinine, cystatin C, KIM-1, and NGAL. The biomarker concentrations that differ at 30 weeks PMA include clusterin, EGF, KIM-1, NGAL, and OPN. Only OPN concentrations differ at 34 weeks PMA.

The biomarkers concentration which change over time for the 24, 25, 26, and 27 week GA groups are summarized in Table 5. The biomarkers which differ by sex were  $\alpha$ GST, B2M, clusterin, cystatin C, KIM-1 and NGAL, while the rate of change over time by sex differed in albumin, B2M, cystatin C and OPN. No differences were seen by treatment for any biomarkers. Correlation analysis between biomarkers

The biomarker concentration correlations with one another are shown in figure 5.

## Discussion

This ancillary study reports on 10 urine biomarkers and their respective biomarker/cr ratios in 750 extremely low GA neonates without severe AKI. We report how these biomarkers differ at birth by GA, sex and Epo status. We report the trends over both chronological time and PMA by GA, and sex. We found that some biomarker concentrations increase while others decrease over the first 30 days. Although all 10 biomarkers concentrations differ at birth by the GA, 3/10 urine biomarkers were no longer different at 27 weeks PMA; 5/10 were no longer different by 30 weeks PMA, and only 1 (OPN) differed by GA at 34 weeks PMA. This suggest that that the developmental changes leading to differences in biomarkers at birth, become similar by 34 weeks PMA irrespective of the degree of prematurity at birth. About half of the biomarker concentrations differed by sex, and 4/10 showed different rates of change over time between males vs. females. Finally, we did not see any changes in urine biomarkers by treatment arm (Epo vs. placebo). No major differences were seen in these patterns when we evaluate the biomarker/cr ratios.

Nephrogenesis begins at the fifth week of gestation and continues until 34-36 weeks.<sup>36</sup> Study of the urine proteome may lend insights into the developmental process that are occurring at an individual level. Some urine biomarkers (i.e. B2M, Clusterin, KIM-1, NGAL) decrease over time, presumably due to maturation of tubular reabsorption capacity of these proteins which occur with tubular maturity. In contrast, we found that some urine

biomarkers (i.e. EGF and UMOD) rise over time, presumably due to increased tubular mass. Further evaluation of these relationships and the mechanisms which underlie these changes are beyond the scope of this analysis. Further research is needed to provide important insights into how urine tubular / glomerular development can be tracked with the urine proteome.

In 2015, Saedi et al. published data on many of the same biomarkers over the first 2 postnatal weeks in 81 premature neonates without AKI from a single-center cohort.<sup>21</sup> They concluded that many of these urine biomarkers are affected by sex and change over time from birth. The current study corroborates these findings in a larger, multi-center cohort and shows differences by smaller GA intervals and provides insights on how these biomarker concentrations change over a longer period of time. In addition, because we have urine collection for many weeks after birth, this report provides insights about changes by maturation as we evaluate biomarkers at the 27, 30 and 34 weeks PMA timepoints. This study provides insights as we compare biomarker concentrations at 27 week PMA in urine from day 21 postnatal in 24 week GA neonate vs urine from day 7 postnatal in a 26 week GA neonate.

The strengths of this study include the collection of a large sample size from multiple centers and large number of urine specimens collected at different timepoints over the first months of life. Despite these strengths, we acknowledge several important limitations. First, not all neonates had urine collected at the designated timepoints. Second, most of the urine measurements were collected using cotton balls, which could alter urine biomarker concentrations.<sup>37</sup> Third, because not all neonates had SCr measured every day, we may have missed some neonates who had severe AKI and would have otherwise been excluded from this study. Forth, we acknowledge that the CV% for B2M was poor making inferences about the B2M analysis questionable. For B2M primarily - but also for other biomarkers, values had to be imputed if they were above or below the level of detection. Finally, we recognize that other variables (for example co-morbidities) can affect urine biomarker changes and that changes may be due to other factors besides GA and sex.

Overall, this study suggests that urine biomarkers differ to varying degree by GA, postnatal age, and sex. These differences should be considered in planning, analyzing, and interpreting urine biomarker studies in premature neonates. Although this may not be an issue when evaluating extreme biomarker values (for example as one detects an episode of AKI with a biomarker that rises 5-fold when AKI occurs), it could create complexity for clinicians and researchers as they evaluate whether mild or modest biomarker values are 'abnormal'. To address this issue, evaluation for changes from baseline and/or trends in biomarker concentrations over time (as opposed to evaluation of a single value) may better accomplish the clinical / research goals. Alternatively, evaluation of how the biomarker differs from the expected normative values (for PMA) could be used. Furthermore, these data suggest that there is a 'normalization' of the biomarker patterns by 34 weeks PMA, regardless of the degree of prematurity at birth. This suggests a maturation of tubular proteins secretion/reabsorption normalization over time. How these important questions apply to premature neonates > 28 weeks GA and term neonates need to be evaluated.

## Acknowledgements

We would like to thank Lynn Dill, RN and Emily Pao for their assistance in coordinating the REPaReD study, and to Dana Pass for preparation of the manuscript.

We would like to thank the additional primary investigators, co-investigators, clinicians, research personnel, study team, and families who participated in the PENUT study.

### Financial Support

Recombinant Erythropoietin for Protection of Infant Renal Disease (REPaReD) Study is an NIH NIDDK funded (R01 DK103608) ancillary study designed to look at kidney outcome in patients enrolled in the Preterm Erythropoietin Neuroprotection Trial (PENUT trial) which is an NIH NINDS funded (U01 NS077953, U01 NS077955) trial. Urine creatinine was run at the UAB AKI O'Brien Center core (NIH P30-DK079337). The [clinicaltrials.gov](https://clinicaltrials.gov) identifier is [NCT01378273](https://clinicaltrials.gov/ct2/show/study/NCT01378273).

### Role of funding sources

Funding sources for this study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

## References

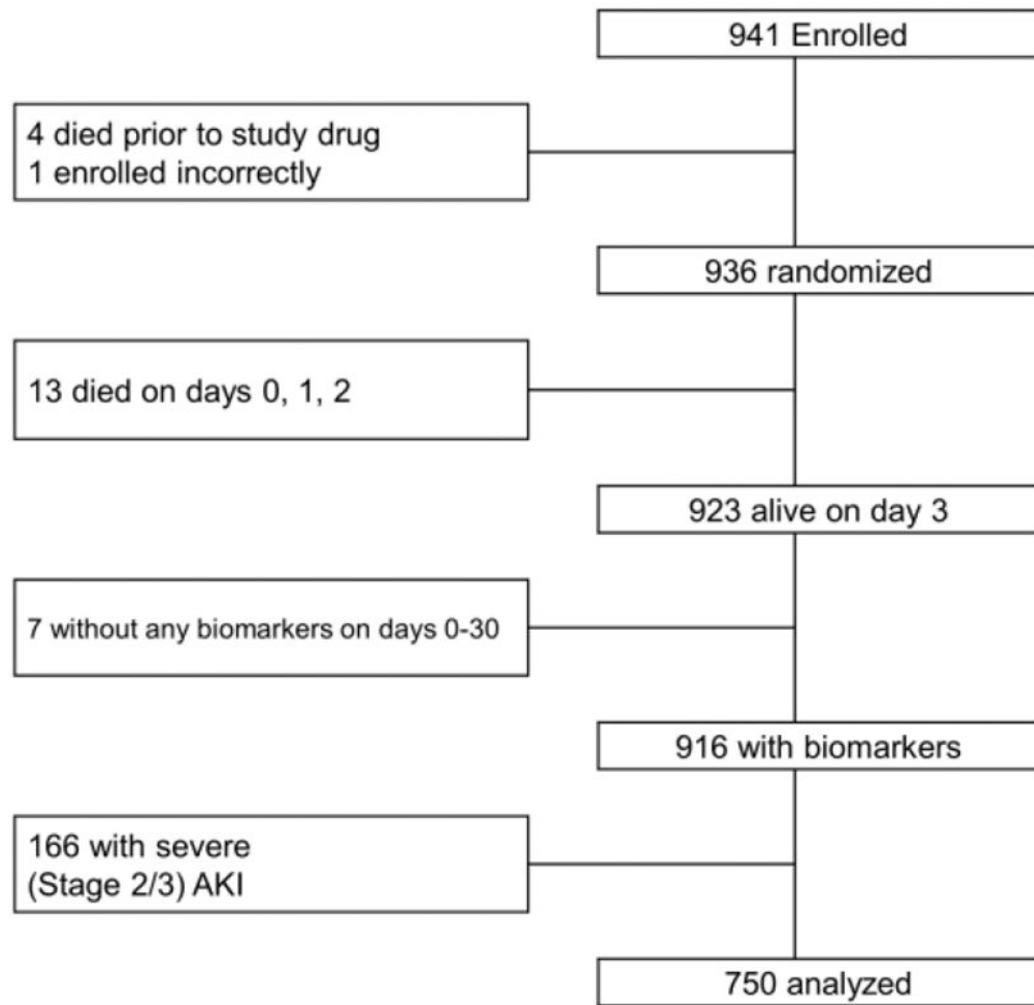
1. Jetton JG et al. Incidence and Outcomes of Neonatal Acute Kidney Injury (Awaken): A Multicentre, Multinational, Observational Cohort Study. *Lancet Child Adolesc Health* 1, 184–194 (2017). [PubMed: 29732396]
2. Koralkar R et al. Acute Kidney Injury Reduces Survival in Very Low Birth Weight Infants. *Pediatr Res* 69, 354–358 (2011). [PubMed: 21178824]
3. Askenazi DJ, Griffin R, McGwin G, Carlo W & Ambalavanan N Acute Kidney Injury Is Independently Associated with Mortality in Very Low Birthweight Infants: A Matched Case-Control Analysis. *Pediatr Nephrol* 24, 991–997 (2009). [PubMed: 19238451]
4. Askenazi DJ et al. Prevalence of Acute Kidney Injury (Aki) in Extremely Low Gestational Age Neonates (Elgan). *Pediatr Nephrol* (2020).
5. Goldstein SL Acute Kidney Injury in Children: Prevention, Treatment and Rehabilitation. *Contrib Nephrol* 174, 163–172 (2011). [PubMed: 21921621]
6. Soni SS, Ronco C, Katz N & Cruz DN Early Diagnosis of Acute Kidney Injury: The Promise of Novel Biomarkers. *Blood Purif* 28, 165–174 (2009). [PubMed: 19590184]
7. Ostermann M, Philips BJ & Forni LG Clinical Review: Biomarkers of Acute Kidney Injury: Where Are We Now? *Crit Care* 16, 233 (2012). [PubMed: 23014769]
8. Askenazi DJ et al. Baseline Values of Candidate Urine Acute Kidney Injury (Aki) Biomarkers Vary by Gestational Age in Premature Infants. *Pediatr Res* (2011).
9. Ahn YH, Lee J, Chun J, Jun YH & Sung TJ Urine Biomarkers for Monitoring Acute Kidney Injury in Premature Infants. *Kidney Res Clin Pract* 39, 284–294 (2020). [PubMed: 32839353]
10. Kamianowska M, Szczepanski M & Wasilewska A Tubular and Glomerular Biomarkers of Acute Kidney Injury in Newborns. *Curr Drug Metab* 20, 332–349 (2019). [PubMed: 30907310]
11. Sellmer A et al. Urinary Neutrophil Gelatinase-Associated Lipocalin in the Evaluation of Patent Ductus Arteriosus and Aki in Very Preterm Neonates: A Cohort Study. *BMC Pediatr* 17, 7 (2017). [PubMed: 28068947]
12. Tanigasalam V, Bhat BV, Adhisivam B, Sridhar MG & Harichandrakumar KT Predicting Severity of Acute Kidney Injury in Term Neonates with Perinatal Asphyxia Using Urinary Neutrophil Gelatinase Associated Lipocalin. *Indian J Pediatr* 83, 1374–1378 (2016). [PubMed: 27299341]
13. Hanna M et al. Early Urinary Biomarkers of Acute Kidney Injury in Preterm Infants. *Pediatr Res* 80, 218–223 (2016). [PubMed: 27055185]
14. Chen CN et al. Urinary Neutrophil Gelatinase-Associated Lipocalin Levels in Neonates. *Pediatr Neonatol* 57, 207–212 (2016). [PubMed: 26563762]

15. Askenazi DJ et al. Acute Kidney Injury Urine Biomarkers in Very Low-Birth-Weight Infants. *Clin J Am Soc Nephrol* 11, 1527–1535 (2016). [PubMed: 27471253]
16. Askenazi DJ et al. Urine Biomarkers Predict Acute Kidney Injury in Newborns. *J Pediatr* 161, 270–275 e271 (2012). [PubMed: 22424940]
17. Askenazi DJ et al. Urine Biomarkers Predict Acute Kidney Injury and Mortality in Very Low Birth Weight Infants. *J Pediatr* 159, 907–912 e901 (2011). [PubMed: 21784446]
18. Balena-Borneman J et al. Biomarkers Associated with Bronchopulmonary Dysplasia/Mortality in Premature Infants. *Pediatr Res* 81, 519–525 (2017). [PubMed: 27893721]
19. Shima Y, Kumasaka S & Nishimaki S Urinary Beta2-Microglobulin and Bronchopulmonary Dysplasia: Trends in Preterm Infants. *Pediatr Int* 59, 1169–1173 (2017). [PubMed: 28833913]
20. Askenazi DJ et al. Baseline Values of Candidate Urine Acute Kidney Injury Biomarkers Vary by Gestational Age in Premature Infants. *Pediatr Res* 70, 302–306 (2011). [PubMed: 21646940]
21. Saeidi B et al. Impact of Gestational Age, Sex, and Postnatal Age on Urine Biomarkers in Premature Neonates. *Pediatr Nephrol* 30, 2037–2044 (2015). [PubMed: 26001700]
22. Lavery AP et al. Urinary Ngal in Premature Infants. *Pediatr Res* 64, 423–428 (2008). [PubMed: 18552711]
23. Huynh TK et al. Reference Values of Urinary Neutrophil Gelatinase-Associated Lipocalin in Very Low Birth Weight Infants. *Pediatr Res* 66, 528–532 (2009). [PubMed: 19680166]
24. Bennett MR, Nehus E, Haffner C, Ma Q & Devarajan P Pediatric Reference Ranges for Acute Kidney Injury Biomarkers. *Pediatr Nephrol* (2014).
25. Weber A, Harrison TM, Sinnott L, Shoben A & Steward D Plasma and Urinary Oxytocin Trajectories in Extremely Premature Infants During Nicu Hospitalization. *Biol Res Nurs* 19, 549–558 (2017). [PubMed: 28699358]
26. Nakata Y, Okada H, Itoh S & Kusaka T Developmental Changes in Urinary Coproporphyrin Ratio in Premature Infants. *Pediatr Int* 62, 65–69 (2020). [PubMed: 31628881]
27. Juul SE, Mayock DE, Comstock BA & Heagerty PJ Neuroprotective Potential of Erythropoietin in Neonates; Design of a Randomized Trial. *Matern Health Neonatol Perinatol* 1, 27 (2015). [PubMed: 27057344]
28. Juul SE et al. A Randomized Trial of Erythropoietin for Neuroprotection in Preterm Infants. *N Engl J Med* 382, 233–243 (2020). [PubMed: 31940698]
29. Kellum JA, Lameire N & Group KAGW Diagnosis, Evaluation, and Management of Acute Kidney Injury: A Kdigo Summary (Part 1). *Crit Care* 17, 204 (2013). [PubMed: 23394211]
30. Thayyil S, Sheik S, Kempley ST & Sinha A A Gestation- and Postnatal Age-Based Reference Chart for Assessing Renal Function in Extremely Premature Infants. *J Perinatol* 28, 226–229 (2008). [PubMed: 18288122]
31. Bateman DA et al. Serum Creatinine Concentration in Very-Low-Birth-Weight Infants from Birth to 34-36 Wk Postmenstrual Age. *Pediatric research* 77, 696–702 (2015). [PubMed: 25675426]
32. Engle WA, American Academy of Pediatrics Committee on, F. & Newborn. Age Terminology During the Perinatal Period. *Pediatrics* 114, 1362–1364 (2004). [PubMed: 15520122]
33. Amaral Pedrosa L et al. Acute Kidney Injury Biomarkers in the Critically Ill. *Clin Chim Acta* 508, 170–178 (2020). [PubMed: 32413402]
34. Devarajan P The Current State of the Art in Acute Kidney Injury. *Front Pediatr* 8, 70 (2020). [PubMed: 32257978]
35. Zeger SL & Liang KY Longitudinal Data Analysis for Discrete and Continuous Outcomes. *Biometrics* 42, 121–130 (1986). [PubMed: 3719049]
36. Hinchliffe SA, Sargent PH, Howard CV, Chan YF & van Velzen D Human Intrauterine Renal Growth Expressed in Absolute Number of Glomeruli Assessed by the Disector Method and Cavalieri Principle. *Lab Invest* 64, 777–784 (1991). [PubMed: 2046329]
37. Boohaker L et al. Absorbent Materials to Collect Urine Can Affect Proteomics and Metabolomic Biomarker Concentrations. *Clin Chem Lab Med* 57, e134–e137 (2019). [PubMed: 30375345]

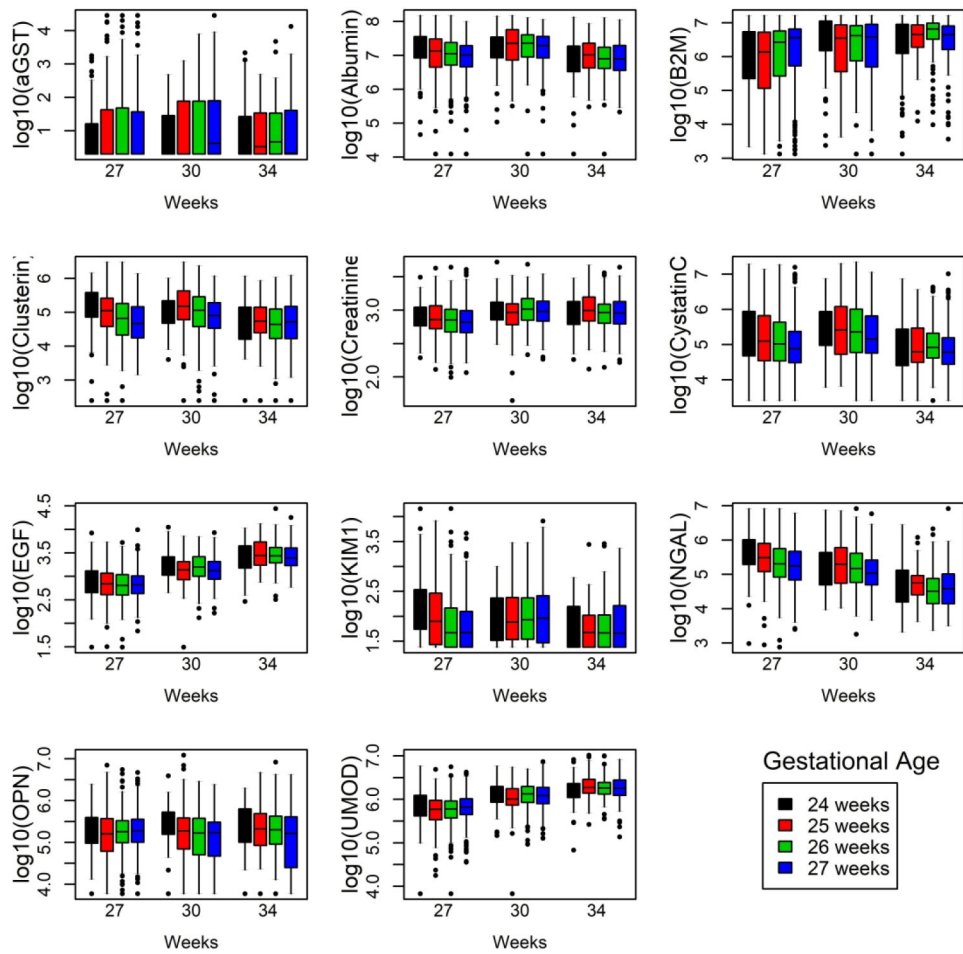


**Impact Statement:**

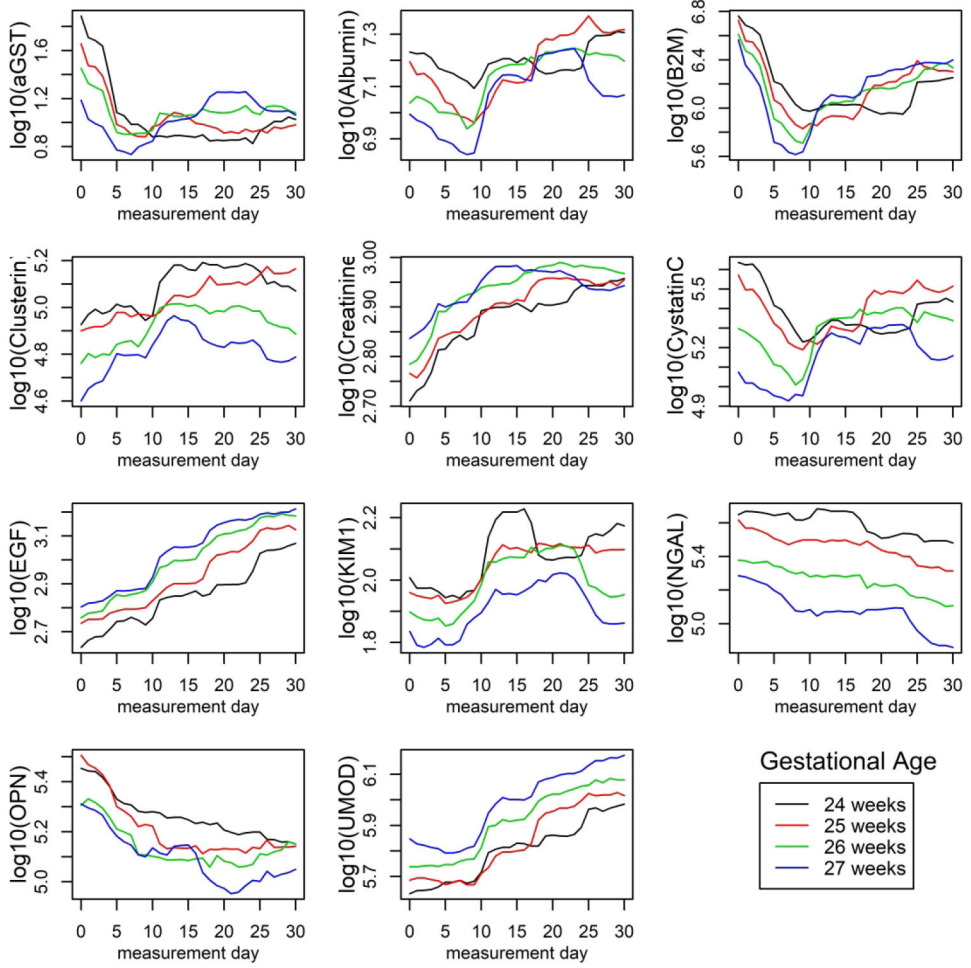
1. Urine biomarker concentrations differ by gestational age (GA) at birth.
2. Some urine biomarkers increase, while others decrease, over the first 30 postnatal days.
3. Most urine biomarkers differ by GA at 27 weeks post-menstrual age (PMA) but are similar by 34 weeks PMA.
4. Some urine biomarkers vary by sex in premature neonates.
5. Urine biomarkers did not differ between neonates randomized to placebo vs. erythropoietin (Epo).



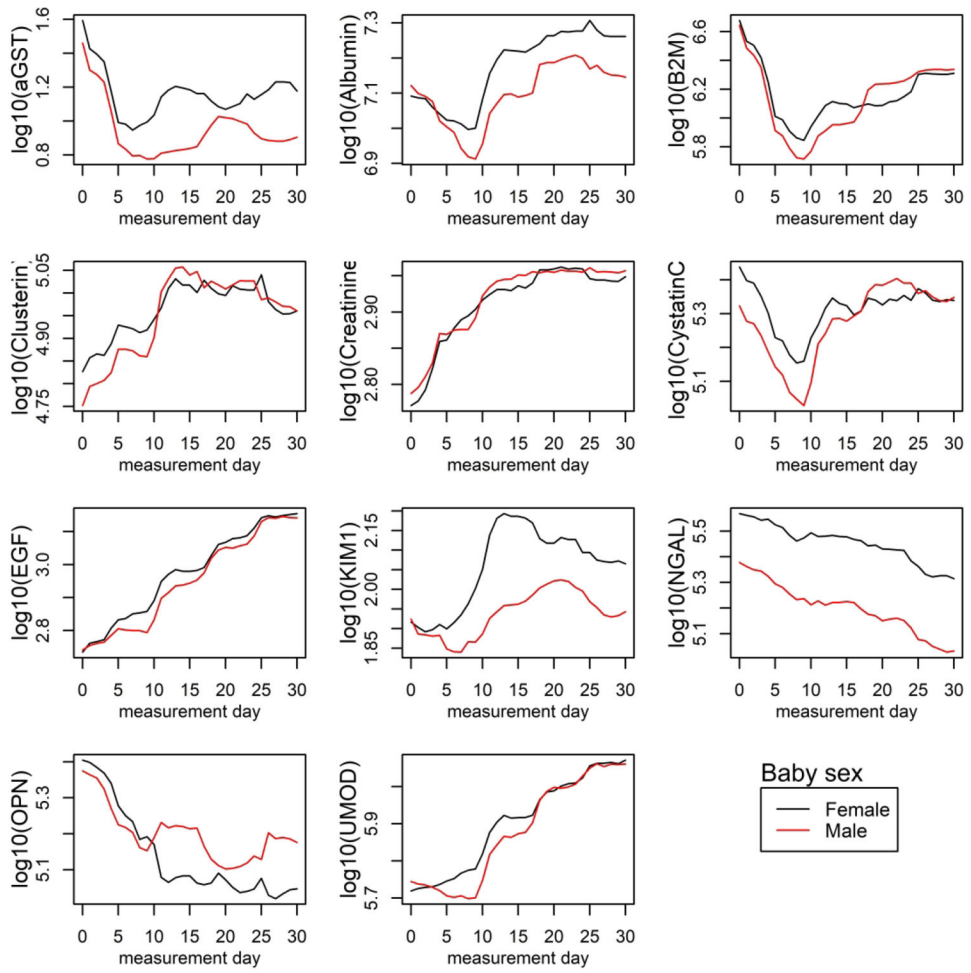
**Figure 1:**  
Consort diagram showing the reasons for exclusion for this analysis from the 940 enrolled into the PENUT trial.



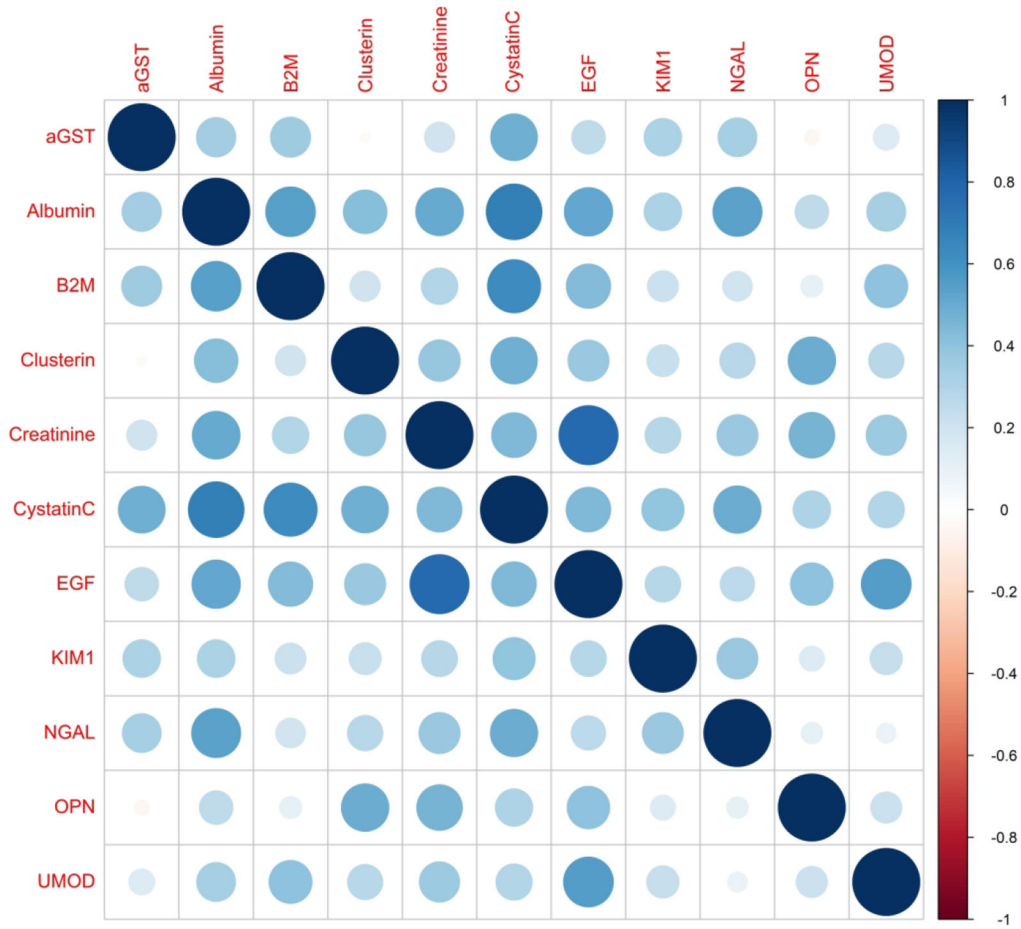
**Figure 2:** Mean log<sub>10</sub> urine biomarker concentrations over postnatal days by GA groups (24, 25, 26, 27 weeks GA) using a 7-day rolling mean.



**Figure 3:** Median (IQR) log<sub>10</sub> urine biomarker concentrations by GA groups at 27, 30 and 34 weeks post-menstrual age (PMA) by groups (24, 25, 26, 27 weeks GA).



**Figure 4:** Mean log<sub>10</sub> urine biomarker concentrations over postnatal days for males vs. females using a 7-day rolling mean.



**Figure 5:**  
Correlation between the 10 biomarkers and one another

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript



**Table 1a:**

## Demographics by gestational age groups

	24 weeks	25 weeks	26 weeks	27 weeks
n	163	188	187	212
Sex, n (%)				
Male	88 (54.0%)	91 (48.4%)	96 (51.3%)	105 (49.5%)
Female	75 (46.0%)	97 (51.6%)	91 (48.7%)	107 (50.5%)
Birth weight (g), mean (sd)	667.2 (113.0)	755.9 (130.0)	879.9 (164.3)	936.9 (190.1)
Birth length (cm), mean (sd)	30.9 (2.2)	32.2 (2.5)	34 (2.4)	34.8 (2.7)
Size for GA, n (%)				
Large	25 (15.3%)	18 (9.6%)	30 (16.0%)	15 (7.1%)
Average	129 (79.1%)	161 (85.6%)	145 (77.5%)	173 (81.6%)
Small	9 (5.5%)	9 (4.8%)	12 (6.4%)	24 (11.3%)
Mother's Race, n (%)				
White	97 (59.5%)	115 (61.2%)	115 (61.5%)	158 (74.5%)
Black	55 (33.7%)	54 (28.7%)	46 (24.6%)	40 (18.9%)
Other	6 (3.7%)	12 (6.4%)	17 (9.1%)	13 (6.1%)
Unknown	5 (3.1%)	7 (3.7%)	9 (4.8%)	1 (0.5%)
Ethnicity				
Hispanic	33 (20.2%)	36 (19.1%)	42 (22.5%)	46 (21.7%)
Not hispanic	129 (79.1%)	149 (79.3%)	143 (76.5%)	163 (76.9%)
Unknown	1 (0.6%)	3 (1.6%)	2 (1.1%)	3 (1.4%)
Apgar 1 minute, median (IQR)	3 (2, 5)	3 (1, 6)	4 (2, 6)	5 (3, 6)
Apgar 5 minute, median (IQR)	6 (4, 7)	7 (4.75, 7)	7 (6, 8)	7 (6, 8)
Birth OFC, mean (sd)	21.5 (1.2)	22.8 (1.8)	23.8 (1.6)	24.5 (1.5)
# of fetuses, mean (sd)	1.3 (0.6)	1.3 (0.5)	1.2 (0.4)	1.3 (0.5)
Treatment				
Epo	73 (44.8%)	96 (51.1%)	80 (42.8%)	124 (58.5%)
Placebo	90 (55.2%)	92 (48.9%)	107 (57.2%)	88 (41.5%)

**Table 1b:**

## Demographics by gender

	Female	Male
n	370	380
Gestational Age, n (%)		
24 weeks	75 (20.3%)	88 (23.2%)
25 weeks	97 (26.2%)	91 (23.9%)
26 weeks	91 (24.6%)	96 (25.3%)
27 weeks	107 (28.9%)	105 (27.6%)
Birth weight (g), mean (sd)	793.3 (177.9)	843.5 (191.2)
Birth length (cm), mean (sd)	32.8 (3.0)	33.4 (2.8)
Size for GA, n (%)		
Large	27 (7.3%)	61 (16.1%)
Average	312 (84.3%)	296 (77.9%)
Small	31 (8.4%)	23 (6.1%)
Mother's Race, n (%)		
White	234 (63.2%)	251 (66.1%)
Black	0 (0.0%)	0 (0.0%)
Other	126 (34.1%)	117 (30.8%)
Unknown	10 (2.7%)	12 (3.2%)
Ethnicity		
Hispanic	75 (20.3%)	82 (21.6%)
Not hispanic	290 (78.4%)	294 (77.4%)
Unknown	5 (1.4%)	4 (1.1%)
Apgar 1 minute, median (IQR)	4 (2, 6)	4 (2, 6)
Apgar 5 minute, median (IQR)	7 (5, 8)	7 (5, 8)
Birth OFC, mean (sd)	23 (1.8)	23.5 (2.0)
# of fetuses, mean (sd)	1.3 (0.5)	1.3 (0.6)
Treatment		
Epo	179 (48.4%)	194 (51.1%)
Placebo	191 (51.6%)	186 (48.9%)

**Table 2:**

Predicted mean (SD) biomarker and biomarker/cr at birth and slope for % change per week by GA

	Biomarker (pg/ml)			Biomarker / Cr (unitless)	
	Birth fitted value	% change from birth per week		Birth fitted value	% change from birth per week
<b>αGST</b>	**	**	aGST / Cr	**	**
GA 24	25.9 (20.4, 33.0)	-23.4 (-28.8, -17.7)	GA 24	$4.15 \times 10^{-7}$ ( $3.22 \times 10^{-7}$ , $5.35 \times 10^{-7}$ )	-31.6 (-36.6, -26.3)
GA 25	18.5 (14.8, 23.1)	-14.1 (-20.3, -7.33)	GA 25	$2.71 \times 10^{-7}$ ( $2.14 \times 10^{-7}$ , $3.44 \times 10^{-7}$ )	-22.5 (-28.5, -16.1)
GA 26	16.3 (12.9, 20.7)	-10.3 (-19.0, -0.65)	GA 26	$2.28 \times 10^{-7}$ ( $1.77 \times 10^{-7}$ , $2.93 \times 10^{-7}$ )	-20.8 (-28.7, -11.9)
GA 27	8.64 (7.07, 10.6)	13.2 (2.64, 24.8)	GA 27	$1.11 \times 10^{-7}$ ( $0.90 \times 10^{-7}$ , $1.37 \times 10^{-7}$ )	2.72 (-7.13, 13.6)
Albumin	**	**	Albumin / Cr	**	**
GA 24	$1.54 \times 10^7$ ( $1.39 \times 10^7$ , $1.71 \times 10^7$ )	1.31 (-2.30, 5.05)	GA 24	0.25 (0.22, 0.27)	-9.56 (-12.7, -6.37)
GA 25	$1.21 \times 10^7$ ( $1.08 \times 10^7$ , $1.35 \times 10^7$ )	9.73 (5.76, 13.9)	GA 25	0.18 (0.16, 0.20)	-1.07 (-4.65, 2.65)
GA 26	$1.03 \times 10^7$ ( $0.91 \times 10^7$ , $1.16 \times 10^7$ )	14.4 (8.40, 20.8)	GA 26	0.14 (0.13, 0.16)	1.06 (-3.69, 6.05)
GA 27	$0.82 \times 10^7$ ( $0.73 \times 10^7$ , $0.92 \times 10^7$ )	17.7 (11.3, 24.5)	GA 27	0.10 (0.09, 0.12)	7.12 (1.72, 12.8)
B2M	**	*	B2M / Cr	**	*
GA 24	$2.25 \times 10^6$ ( $1.90 \times 10^6$ , $2.67 \times 10^6$ )	-7.99 (-13.3, -2.35)	GA 24	$3.61 \times 10^{-2}$ ( $3.00 \times 10^{-2}$ , $4.34 \times 10^{-2}$ )	-17.9 (-22.7, -12.7)
GA 25	$1.74 \times 10^6$ ( $1.45 \times 10^6$ , $2.09 \times 10^6$ )	0.89 (-5.00, 7.14)	GA 25	$2.55 \times 10^{-2}$ ( $2.09 \times 10^{-2}$ , $3.11 \times 10^{-2}$ )	-9.04 (-14.7, -3.03)
GA 26	$1.63 \times 10^6$ ( $1.34 \times 10^6$ , $1.99 \times 10^6$ )	-3.31 (-11.51, 5.65)	GA 26	$2.28 \times 10^{-2}$ ( $1.84 \times 10^{-2}$ , $2.82 \times 10^{-2}$ )	-14.6 (-22.1, -6.41)
GA 27	$1.18 \times 10^6$ ( $0.96 \times 10^6$ , $1.45 \times 10^6$ )	8.84 (-0.72, 19.3)	GA 27	$1.52 \times 10^{-2}$ ( $1.22 \times 10^{-2}$ , $1.89 \times 10^{-2}$ )	-1.34 (-10.4, 8.68)
Clusterin	**		Clusterin/ Cr	**	
GA 24	$1.03 \times 10^5$ ( $0.91 \times 10^5$ , $1.17 \times 10^5$ )	3.99 (-0.34, 8.51)	GA 24	$1.66 \times 10^{-3}$ ( $1.46 \times 10^{-3}$ , $1.88 \times 10^{-3}$ )	-7.16 (-10.9, -3.30)
GA 25	$0.90 \times 10^5$ ( $0.78 \times 10^5$ , $1.03 \times 10^5$ )	4.73 (-0.92, 10.7)	GA 25	$1.32 \times 10^{-3}$ ( $1.15 \times 10^{-3}$ , $1.51 \times 10^{-3}$ )	-5.58 (-10.5, -0.43)
GA 26	$0.68 \times 10^5$ ( $0.58 \times 10^5$ , $0.79 \times 10^5$ )	8.71 (1.24, 16.7)	GA 26	$0.95 \times 10^{-3}$ ( $0.82 \times 10^{-3}$ , $1.10 \times 10^{-3}$ )	-3.99 (-10.2, 2.70)
GA 27	$0.50 \times 10^5$ ( $0.44 \times 10^5$ , $0.57 \times 10^5$ )	12.6 (5.32, 20.3)	GA 27	$0.64 \times 10^{-3}$ ( $0.57 \times 10^{-3}$ , $0.73 \times 10^{-3}$ )	2.27 (-3.85, 8.77)
Cystatin C	**	**	Cystatin C / Cr	**	**
GA 24	$3.08 \times 10^5$ ( $2.59 \times 10^5$ , $3.66 \times 10^5$ )	-7.89 (-12.8, -2.70)	GA 24	$4.93 \times 10^{-3}$ ( $4.13 \times 10^{-3}$ , $5.89 \times 10^{-3}$ )	-17.8 (-22.1, -13.2)
GA 25	$2.47 \times 10^5$ ( $2.08 \times 10^5$ , $2.93 \times 10^5$ )	0.23 (-5.36, 6.15)	GA 25	$3.62 \times 10^{-3}$ ( $3.03 \times 10^{-3}$ , $4.32 \times 10^{-3}$ )	-9.63 (-14.7, -4.29)
GA 26	$1.56 \times 10^5$ ( $1.30 \times 10^5$ , $1.87 \times 10^5$ )	11.3 (2.74, 20.6)	GA 26	$2.17 \times 10^{-3}$ ( $1.80 \times 10^{-3}$ , $2.62 \times 10^{-3}$ )	-1.70 (-9.07, 6.26)

	Biomarker (pg/ml)		% change from birth per week	Biomarker / Cr (unitless)		% change from birth per week
	Birth fitted value	**		Birth fitted value	*	
GA 27	0.98 x 10 <sup>5</sup> (0.84 x 10 <sup>5</sup> , 1.14 x 10 <sup>5</sup> )	**	18.3 (9.96, 27.3)	GA 27	1.25 x 10 <sup>-3</sup> (1.07 x 10 <sup>-3</sup> , 1.47 x 10 <sup>-3</sup> )	7.62 (0.17, 15.6)
EGF				EGF / Cr	*	*
GA 24	4.35 x 10 <sup>2</sup> (4.05 x 10 <sup>2</sup> , 4.67 x 10 <sup>2</sup> )		26.2 (23.3, 29.1)	GA 24	6.97 x 10 <sup>-6</sup> (6.61 x 10 <sup>-6</sup> , 7.35 x 10 <sup>-6</sup> )	12.7 (10.9, 14.5)
GA 25	5.17 x 10 <sup>2</sup> (4.84 x 10 <sup>2</sup> , 5.52 x 10 <sup>2</sup> )		26.2 (23.2, 29.2)	GA 25	7.58 x 10 <sup>-6</sup> (7.18 x 10 <sup>-6</sup> , 8.00 x 10 <sup>-6</sup> )	13.7 (11.7, 15.8)
GA 26	5.71 x 10 <sup>2</sup> (5.30 x 10 <sup>2</sup> , 6.15 x 10 <sup>2</sup> )		29.2 (25.3, 33.3)	GA 26	7.97 x 10 <sup>-6</sup> (7.53 x 10 <sup>-6</sup> , 8.43 x 10 <sup>-6</sup> )	14.1 (11.6, 16.7)
GA 27	6.16 x 10 <sup>2</sup> (5.78 x 10 <sup>2</sup> , 6.57 x 10 <sup>2</sup> )		29.8 (25.9, 33.8)	GA 27	7.90 x 10 <sup>-6</sup> (7.51 x 10 <sup>-6</sup> , 8.32 x 10 <sup>-6</sup> )	17.9 (15.3, 20.6)
KIM-1		**		KIM-1 / Cr	**	
GA 24	1.06 x 10 <sup>2</sup> (0.93 x 10 <sup>2</sup> , 1.20 x 10 <sup>2</sup> )		2.51 (-1.52, 6.72)	GA 24	1.69 x 10 <sup>-6</sup> (1.49 x 10 <sup>-6</sup> , 1.92 x 10 <sup>-6</sup> )	-8.48 (-11.9, -4.89)
GA 25	0.93 x 10 <sup>2</sup> (0.83 x 10 <sup>2</sup> , 1.04 x 10 <sup>2</sup> )		6.05 (1.92, 10.4)	GA 25	1.36 x 10 <sup>-6</sup> (1.22 x 10 <sup>-6</sup> , 1.52 x 10 <sup>-6</sup> )	-4.39 (-8.12, -0.51)
GA 26	0.82 x 10 <sup>2</sup> (0.72 x 10 <sup>2</sup> , 0.93 x 10 <sup>2</sup> )		8.02 (2.17, 14.2)	GA 26	1.15 x 10 <sup>-6</sup> (1.01 x 10 <sup>-6</sup> , 1.30 x 10 <sup>-6</sup> )	-4.61 (-9.68, 0.75)
GA 27	0.65 x 10 <sup>2</sup> (0.59 x 10 <sup>2</sup> , 0.73 x 10 <sup>2</sup> )		10.4 (4.44, 16.7)	GA 27	0.83 x 10 <sup>-6</sup> (0.75 x 10 <sup>-6</sup> , 0.93 x 10 <sup>-6</sup> )	0.36 (-5.04, 6.07)
NGAL		**		NGAL / Cr	**	
GA 24	5.50 x 10 <sup>5</sup> (4.81 x 10 <sup>5</sup> , 6.28 x 10 <sup>5</sup> )		-17.6 (-21.3, -13.7)	GA 24	8.81 x 10 <sup>-3</sup> (7.70 x 10 <sup>-3</sup> , 10.1 x 10 <sup>-3</sup> )	-26.4 (-29.7, -23.0)
GA 25	3.88 x 10 <sup>5</sup> (3.38 x 10 <sup>5</sup> , 4.46 x 10 <sup>5</sup> )		-14.6 (-18.5, -10.4)	GA 25	5.69 x 10 <sup>-3</sup> (4.95 x 10 <sup>-3</sup> , 6.55 x 10 <sup>-3</sup> )	-23.0 (-26.6, -19.1)
GA 26	2.45 x 10 <sup>5</sup> (2.11 x 10 <sup>5</sup> , 2.84 x 10 <sup>5</sup> )		-13.4 (-18.8, -7.61)	GA 26	3.41 x 10 <sup>-3</sup> (2.95 x 10 <sup>-3</sup> , 3.94 x 10 <sup>-3</sup> )	-23.5 (-28.2, -18.6)
GA 27	1.77 x 10 <sup>5</sup> (1.55 x 10 <sup>5</sup> , 2.02 x 10 <sup>5</sup> )		-16.5 (-21.7, -11.0)	GA 27	2.27 x 10 <sup>-3</sup> (2.00 x 10 <sup>-3</sup> , 2.58 x 10 <sup>-3</sup> )	-24.0 (-28.6, -19.2)
OPN		*	*	OPN / Cr	**	
GA 24	2.35 x 10 <sup>5</sup> (2.07 x 10 <sup>5</sup> , 2.67 x 10 <sup>5</sup> )		-7.27 (-11.4, -2.98)	GA 24	3.77 x 10 <sup>-3</sup> (3.30 x 10 <sup>-3</sup> , 4.29 x 10 <sup>-3</sup> )	-17.2 (-20.8, -13.5)
GA 25	2.30 x 10 <sup>5</sup> (2.04 x 10 <sup>5</sup> , 2.59 x 10 <sup>5</sup> )		-11.1 (-15.5, -6.37)	GA 25	3.38 x 10 <sup>-3</sup> (2.98 x 10 <sup>-3</sup> , 3.83 x 10 <sup>-3</sup> )	-19.8 (-23.9, -15.6)
GA 26	1.83 x 10 <sup>5</sup> (1.62 x 10 <sup>5</sup> , 2.06 x 10 <sup>5</sup> )		-10.9 (-16.3, -5.25)	GA 26	2.55 x 10 <sup>-3</sup> (2.25 x 10 <sup>-3</sup> , 2.89 x 10 <sup>-3</sup> )	-21.3 (-25.9, -16.4)
GA 27	1.88 x 10 <sup>5</sup> (1.69 x 10 <sup>5</sup> , 2.10 x 10 <sup>5</sup> )		-17.3 (-22.1, -12.1)	GA 27	2.41 x 10 <sup>-3</sup> (2.16 x 10 <sup>-3</sup> , 2.69 x 10 <sup>-3</sup> )	-24.9 (-29.2, -20.2)
UMOD		**		UMOD / Cr	*	
GA 24	4.17 x 10 <sup>5</sup> (3.84 x 10 <sup>5</sup> , 4.54 x 10 <sup>5</sup> )		21.2 (18.2, 24.3)	GA 24	6.69 x 10 <sup>-3</sup> (6.19 x 10 <sup>-3</sup> , 7.23 x 10 <sup>-3</sup> )	8.18 (5.68, 10.8)
GA 25	4.37 x 10 <sup>5</sup> (4.06 x 10 <sup>5</sup> , 4.71 x 10 <sup>5</sup> )		23.6 (20.6, 26.6)	GA 25	6.41 x 10 <sup>-3</sup> (5.95 x 10 <sup>-3</sup> , 6.90 x 10 <sup>-3</sup> )	11.4 (8.73, 14.1)
GA 26	5.10 x 10 <sup>5</sup> (4.70 x 10 <sup>5</sup> , 5.53 x 10 <sup>5</sup> )		25.3 (21.1, 29.6)	GA 26	7.11 x 10 <sup>-3</sup> (6.58 x 10 <sup>-3</sup> , 7.68 x 10 <sup>-3</sup> )	10.7 (7.04, 14.4)
GA 27	6.03 x 10 <sup>5</sup> (5.62 x 10 <sup>5</sup> , 6.47 x 10 <sup>5</sup> )		24.8 (21.0, 28.7)	GA 27	7.74 x 10 <sup>-3</sup> (7.18 x 10 <sup>-3</sup> , 8.35 x 10 <sup>-3</sup> )	13.3 (9.63, 17.1)
Creatinine		**				

	Biomarker (pg/ml)		% change from birth per week	Biomarker / Cr (unitless)		% change from birth per week
	Birth fitted value	% change from birth per week		Birth fitted value	% change from birth per week	
GA 24	6.24 (5.89, 6.61)	12.0 (9.96, 14.1)				
GA 25	6.82 (6.46, 7.20)	10.9 (8.75, 13.1)				
GA 26	7.17 (6.71, 7.66)	13.2 (10.1, 16.4)				
GA 27	7.80 (7.39, 8.23)	10.1 (7.31, 12.9)				

\*\* differences across GA are significant at  $p < 0.001$

\* differences across GA are significant at  $p < 0.05$

**Table 3a:** Means (95% CI) biomarker concentration at specific PMA timepoints by GA groups

	27 week	30 week	34 week	p-value for trend
<b><math>\alpha</math>GST</b>				
GA 24	7.30 (5.31, 10.0)	8.20 (5.44, 12.4)	8.27 (5.17, 13.2)	
GA 25	9.98 (7.21, 13.8)	11.5 (7.07, 18.8)	8.50 (5.63, 12.8)	
GA 26	10.8 (8.56, 13.7)	13.1 (9.26, 18.4)	8.94 (6.46, 12.4)	
GA 27	9.19 (7.54, 11.2)	14.4 (10.4, 19.8)	10.6 (6.84, 16.5)	
Albumin	**			
GA 24	$1.58 \times 10^7$ (1.27 x 10 <sup>7</sup> , 1.96 x 10 <sup>7</sup> )	$1.59 \times 10^7$ (1.18 x 10 <sup>7</sup> , 2.13 x 10 <sup>7</sup> )	$0.63 \times 10^7$ (0.42 x 10 <sup>7</sup> , 0.96 x 10 <sup>7</sup> )	**
GA 25	$0.97 \times 10^7$ (0.77 x 10 <sup>7</sup> , 1.22 x 10 <sup>7</sup> )	$1.80 \times 10^7$ (1.32 x 10 <sup>7</sup> , 2.46 x 10 <sup>7</sup> )	$0.91 \times 10^7$ (0.68 x 10 <sup>7</sup> , 1.22 x 10 <sup>7</sup> )	
GA 26	$0.95 \times 10^7$ (0.82 x 10 <sup>7</sup> , 1.10 x 10 <sup>7</sup> )	$1.81 \times 10^7$ (1.50 x 10 <sup>7</sup> , 2.18 x 10 <sup>7</sup> )	$0.78 \times 10^7$ (0.63 x 10 <sup>7</sup> , 0.98 x 10 <sup>7</sup> )	
GA 27	$0.87 \times 10^7$ (0.78 x 10 <sup>7</sup> , 0.99 x 10 <sup>7</sup> )	$1.55 \times 10^7$ (1.31 x 10 <sup>7</sup> , 1.84 x 10 <sup>7</sup> )	$0.78 \times 10^7$ (0.60 x 10 <sup>7</sup> , 1.01 x 10 <sup>7</sup> )	
B2M	*			
GA 24	$1.02 \times 10^6$ (0.73 x 10 <sup>6</sup> , 1.43 x 10 <sup>6</sup> )	$2.79 \times 10^6$ (1.78 x 10 <sup>6</sup> , 4.39 x 10 <sup>6</sup> )	$1.93 \times 10^6$ (1.13 x 10 <sup>6</sup> , 3.29 x 10 <sup>6</sup> )	*
GA 25	$0.72 \times 10^6$ (0.52 x 10 <sup>6</sup> , 1.02 x 10 <sup>6</sup> )	$1.68 \times 10^6$ (1.04 x 10 <sup>6</sup> , 2.70 x 10 <sup>6</sup> )	$3.21 \times 10^6$ (2.25 x 10 <sup>6</sup> , 4.58 x 10 <sup>6</sup> )	**
GA 26	$1.20 \times 10^6$ (0.95 x 10 <sup>6</sup> , 1.50 x 10 <sup>6</sup> )	$1.95 \times 10^6$ (1.45 x 10 <sup>6</sup> , 2.63 x 10 <sup>6</sup> )	$4.23 \times 10^6$ (3.26 x 10 <sup>6</sup> , 5.50 x 10 <sup>6</sup> )	**
GA 27	$1.52 \times 10^6$ (1.24 x 10 <sup>6</sup> , 1.86 x 10 <sup>6</sup> )	$1.81 \times 10^6$ (1.34 x 10 <sup>6</sup> , 2.44 x 10 <sup>6</sup> )	$2.55 \times 10^6$ (1.72 x 10 <sup>6</sup> , 3.78 x 10 <sup>6</sup> )	*
Clusterin	**	*		
GA 24	$15.4 \times 10^4$ (12.3 x 10 <sup>4</sup> , 19.4 x 10 <sup>4</sup> )	$10.3 \times 10^4$ (7.78 x 10 <sup>4</sup> , 13.7 x 10 <sup>4</sup> )	$4.85 \times 10^4$ (3.28 x 10 <sup>4</sup> , 7.16 x 10 <sup>4</sup> )	**
GA 25	$9.46 \times 10^4$ (7.67 x 10 <sup>4</sup> , 11.7 x 10 <sup>4</sup> )	$13.2 \times 10^4$ (9.03 x 10 <sup>4</sup> , 19.4 x 10 <sup>4</sup> )	$4.69 \times 10^4$ (3.11 x 10 <sup>4</sup> , 7.07 x 10 <sup>4</sup> )	*
GA 26	$6.36 \times 10^4$ (5.41 x 10 <sup>4</sup> , 7.49 x 10 <sup>4</sup> )	$7.98 \times 10^4$ (6.07 x 10 <sup>4</sup> , 10.5 x 10 <sup>4</sup> )	$3.99 \times 10^4$ (2.95 x 10 <sup>4</sup> , 5.39 x 10 <sup>4</sup> )	*
GA 27	$4.85 \times 10^4$ (4.25 x 10 <sup>4</sup> , 5.55 x 10 <sup>4</sup> )	$6.98 \times 10^4$ (5.62 x 10 <sup>4</sup> , 8.66 x 10 <sup>4</sup> )	$4.28 \times 10^4$ (2.96 x 10 <sup>4</sup> , 6.19 x 10 <sup>4</sup> )	
Cystatin C	*			
GA 24	$2.04 \times 10^5$ (1.49 x 10 <sup>5</sup> , 2.80 x 10 <sup>5</sup> )	$2.73 \times 10^5$ (1.87 x 10 <sup>5</sup> , 4.00 x 10 <sup>5</sup> )	$0.79 \times 10^5$ (0.53 x 10 <sup>5</sup> , 1.18 x 10 <sup>5</sup> )	**
GA 25	$1.51 \times 10^5$ (1.14 x 10 <sup>5</sup> , 1.99 x 10 <sup>5</sup> )	$2.99 \times 10^5$ (1.94 x 10 <sup>5</sup> , 4.60 x 10 <sup>5</sup> )	$0.90 \times 10^5$ (0.61 x 10 <sup>5</sup> , 1.32 x 10 <sup>5</sup> )	
GA 26	$1.29 \times 10^5$ (1.05 x 10 <sup>5</sup> , 1.59 x 10 <sup>5</sup> )	$2.35 \times 10^5$ (1.77 x 10 <sup>5</sup> , 3.11 x 10 <sup>5</sup> )	$0.98 \times 10^5$ (0.74 x 10 <sup>5</sup> , 1.28 x 10 <sup>5</sup> )	
GA 27	$0.98 \times 10^5$ (0.83 x 10 <sup>5</sup> , 1.14 x 10 <sup>5</sup> )	$2.01 \times 10^5$ (1.60 x 10 <sup>5</sup> , 2.54 x 10 <sup>5</sup> )	$0.79 \times 10^5$ (0.56 x 10 <sup>5</sup> , 1.13 x 10 <sup>5</sup> )	
EGF		*		



Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

	27 week	30 week	34 week	p-value for trend
GA 24	0.76 x 10 <sup>3</sup> (0.67 x 10 <sup>3</sup> , 0.86 x 10 <sup>3</sup> )	1.69 x 10 <sup>3</sup> (1.43 x 10 <sup>3</sup> , 1.99 x 10 <sup>3</sup> )	2.52 x 10 <sup>3</sup> (2.10 x 10 <sup>3</sup> , 3.02 x 10 <sup>3</sup> )	**
GA 25	0.68 x 10 <sup>3</sup> (0.61 x 10 <sup>3</sup> , 0.76 x 10 <sup>3</sup> )	1.31 x 10 <sup>3</sup> (1.10 x 10 <sup>3</sup> , 1.57 x 10 <sup>3</sup> )	3.08 x 10 <sup>3</sup> (2.60 x 10 <sup>3</sup> , 3.64 x 10 <sup>3</sup> )	**
GA 26	0.65 x 10 <sup>3</sup> (0.60 x 10 <sup>3</sup> , 0.71 x 10 <sup>3</sup> )	1.51 x 10 <sup>3</sup> (1.36 x 10 <sup>3</sup> , 1.69 x 10 <sup>3</sup> )	2.76 x 10 <sup>3</sup> (2.45 x 10 <sup>3</sup> , 3.11 x 10 <sup>3</sup> )	**
GA 27	0.68 x 10 <sup>3</sup> (0.63 x 10 <sup>3</sup> , 0.72 x 10 <sup>3</sup> )	1.32 x 10 <sup>3</sup> (1.21 x 10 <sup>3</sup> , 1.44 x 10 <sup>3</sup> )	2.53 x 10 <sup>3</sup> (2.19 x 10 <sup>3</sup> , 2.94 x 10 <sup>3</sup> )	**
KIM-1	**	**		
GA 24	147.6 (116.8, 186.5)	93.7 (72.7, 120.7)	67.5 (52.6, 86.6)	**
GA 25	106.9 (87.56, 130.5)	106.4 (80.2, 141.3)	65.1 (50.3, 84.4)	*
GA 26	72.5 (63.2, 83.0)	104.9 (86.7, 127)	60.9 (50.0, 74.1)	
GA 27	62.4 (56.4, 69.1)	106.7 (88.2, 129.0)	70.1 (55.1, 89.3)	*
NGAL	**	*		
GA 24	4.17 x 10 <sup>5</sup> (3.30 x 10 <sup>5</sup> , 5.28 x 10 <sup>5</sup> )	1.48 x 10 <sup>5</sup> (1.04 x 10 <sup>5</sup> , 2.10 x 10 <sup>5</sup> )	0.42 x 10 <sup>4</sup> (0.30 x 10 <sup>4</sup> , 0.60 x 10 <sup>4</sup> )	**
GA 25	3.05 x 10 <sup>5</sup> (2.50 x 10 <sup>5</sup> , 3.73 x 10 <sup>5</sup> )	1.96 x 10 <sup>5</sup> (1.39 x 10 <sup>5</sup> , 2.76 x 10 <sup>5</sup> )	0.51 x 10 <sup>4</sup> (0.38 x 10 <sup>4</sup> , 0.67 x 10 <sup>4</sup> )	**
GA 26	2.05 x 10 <sup>5</sup> (1.76 x 10 <sup>5</sup> , 2.40 x 10 <sup>5</sup> )	1.56 x 10 <sup>5</sup> (1.24 x 10 <sup>5</sup> , 1.95 x 10 <sup>5</sup> )	0.36 x 10 <sup>4</sup> (0.28 x 10 <sup>4</sup> , 0.45 x 10 <sup>4</sup> )	**
GA 27	1.77 x 10 <sup>5</sup> (1.55 x 10 <sup>5</sup> , 2.02 x 10 <sup>5</sup> )	1.16 x 10 <sup>5</sup> (0.97 x 10 <sup>5</sup> , 1.38 x 10 <sup>5</sup> )	0.42 x 10 <sup>4</sup> (0.31 x 10 <sup>4</sup> , 0.56 x 10 <sup>4</sup> )	**
OPN	**	**	*	
GA 24	1.72 x 10 <sup>5</sup> (1.39 x 10 <sup>5</sup> , 2.14 x 10 <sup>5</sup> )	2.39 x 10 <sup>5</sup> (1.73 x 10 <sup>5</sup> , 3.29 x 10 <sup>5</sup> )	2.20 x 10 <sup>5</sup> (1.54 x 10 <sup>5</sup> , 3.15 x 10 <sup>5</sup> )	
GA 25	1.32 x 10 <sup>5</sup> (1.07 x 10 <sup>5</sup> , 1.62 x 10 <sup>5</sup> )	1.53 x 10 <sup>5</sup> (1.04 x 10 <sup>5</sup> , 2.25 x 10 <sup>5</sup> )	1.73 x 10 <sup>5</sup> (1.15 x 10 <sup>5</sup> , 2.62 x 10 <sup>5</sup> )	
GA 26	1.71 x 10 <sup>5</sup> (1.52 x 10 <sup>5</sup> , 1.93 x 10 <sup>5</sup> )	1.22 x 10 <sup>5</sup> (0.95 x 10 <sup>5</sup> , 1.57 x 10 <sup>5</sup> )	1.70 x 10 <sup>5</sup> (1.30 x 10 <sup>5</sup> , 2.22 x 10 <sup>5</sup> )	
GA 27	1.84 x 10 <sup>5</sup> (1.66 x 10 <sup>5</sup> , 2.05 x 10 <sup>5</sup> )	1.12 x 10 <sup>5</sup> (0.90 x 10 <sup>5</sup> , 1.40 x 10 <sup>5</sup> )	1.08 x 10 <sup>5</sup> (0.73 x 10 <sup>4</sup> , 1.59 x 10 <sup>5</sup> )	**
UMOD				
GA 24	0.69 x 10 <sup>6</sup> (0.59 x 10 <sup>6</sup> , 0.81 x 10 <sup>6</sup> )	1.28 x 10 <sup>6</sup> (1.07 x 10 <sup>6</sup> , 1.52 x 10 <sup>6</sup> )	1.51 x 10 <sup>6</sup> (1.26 x 10 <sup>6</sup> , 1.81 x 10 <sup>6</sup> )	**
GA 25	0.53 x 10 <sup>6</sup> (0.47 x 10 <sup>6</sup> , 0.61 x 10 <sup>6</sup> )	1.04 x 10 <sup>6</sup> (0.84 x 10 <sup>6</sup> , 1.28 x 10 <sup>6</sup> )	1.95 x 10 <sup>6</sup> (1.64 x 10 <sup>6</sup> , 2.31 x 10 <sup>6</sup> )	**
GA 26	0.57 x 10 <sup>6</sup> (0.52 x 10 <sup>6</sup> , 0.62 x 10 <sup>6</sup> )	1.20 x 10 <sup>6</sup> (1.08 x 10 <sup>6</sup> , 1.35 x 10 <sup>6</sup> )	1.81 x 10 <sup>6</sup> (1.62 x 10 <sup>6</sup> , 2.01 x 10 <sup>6</sup> )	**
GA 27	0.65 x 10 <sup>6</sup> (0.61 x 10 <sup>6</sup> , 0.70 x 10 <sup>6</sup> )	1.18 x 10 <sup>6</sup> (1.07 x 10 <sup>6</sup> , 1.30 x 10 <sup>6</sup> )	1.73 x 10 <sup>6</sup> (1.49 x 10 <sup>6</sup> , 2.01 x 10 <sup>6</sup> )	**
Creatinine	*			
GA 24	7.90 x 10 <sup>2</sup> (7.26 x 10 <sup>2</sup> , 8.62 x 10 <sup>2</sup> )	9.61 x 10 <sup>2</sup> (8.47 x 10 <sup>2</sup> , 10.9 x 10 <sup>2</sup> )	8.53 x 10 <sup>2</sup> (7.40 x 10 <sup>2</sup> , 9.84 x 10 <sup>2</sup> )	
GA 25	7.51 x 10 <sup>2</sup> (6.92 x 10 <sup>2</sup> , 8.17 x 10 <sup>2</sup> )	8.47 x 10 <sup>2</sup> (7.24 x 10 <sup>2</sup> , 9.92 x 10 <sup>2</sup> )	10.1 x 10 <sup>2</sup> (8.52 x 10 <sup>2</sup> , 11.9 x 10 <sup>2</sup> )	**
GA 26	6.97 x 10 <sup>2</sup> (6.54 x 10 <sup>2</sup> , 7.43 x 10 <sup>2</sup> )	9.93 x 10 <sup>2</sup> (9.10 x 10 <sup>2</sup> , 10.8 x 10 <sup>2</sup> )	8.82 x 10 <sup>2</sup> (7.95 x 10 <sup>2</sup> , 9.79 x 10 <sup>2</sup> )	**
GA 27	6.82 x 10 <sup>2</sup> (6.44 x 10 <sup>2</sup> , 7.21 x 10 <sup>2</sup> )	9.49 x 10 <sup>2</sup> (8.83 x 10 <sup>2</sup> , 10.2 x 10 <sup>2</sup> )	8.78 x 10 <sup>2</sup> (7.70 x 10 <sup>2</sup> , 10.0 x 10 <sup>2</sup> )	**

**Table 3b: Means (95% CI) biomarker/creatinine ratio at specific PMA timepoints by GA groups**

	27 week	30 week	34 week	p-value for trend
<b><math>\alpha</math>GST / Cr</b>				
GA 24	8.17 x 10 <sup>-8</sup> (5.95 x 10 <sup>-8</sup> , 11.2 x 10 <sup>-8</sup> )	7.55 x 10 <sup>-8</sup> (4.91 x 10 <sup>-8</sup> , 11.6 x 10 <sup>-8</sup> )	8.58 x 10 <sup>-8</sup> (5.41 x 10 <sup>-8</sup> , 13.6 x 10 <sup>-8</sup> )	
GA 25	11.8 x 10 <sup>-8</sup> (8.45 x 10 <sup>-8</sup> , 16.4 x 10 <sup>-8</sup> )	12.0 x 10 <sup>-8</sup> (7.20 x 10 <sup>-8</sup> , 20.1 x 10 <sup>-8</sup> )	7.48 x 10 <sup>-8</sup> (5.01 x 10 <sup>-8</sup> , 11.2 x 10 <sup>-8</sup> )	
GA 26	13.8 x 10 <sup>-8</sup> (10.8 x 10 <sup>-8</sup> , 17.6 x 10 <sup>-8</sup> )	11.6 x 10 <sup>-8</sup> (8.34 x 10 <sup>-8</sup> , 16.3 x 10 <sup>-8</sup> )	8.97 x 10 <sup>-8</sup> (6.45 x 10 <sup>-8</sup> , 12.5 x 10 <sup>-8</sup> )	
GA 27	11.9 x 10 <sup>-8</sup> (9.66 x 10 <sup>-8</sup> , 14.7 x 10 <sup>-8</sup> )	13.4 x 10 <sup>-8</sup> (9.68 x 10 <sup>-8</sup> , 18.6 x 10 <sup>-8</sup> )	10.7 x 10 <sup>-8</sup> (6.90 x 10 <sup>-8</sup> , 16.6 x 10 <sup>-8</sup> )	
<b>Albumin / Cr</b>				
GA 24	1.76 x 10 <sup>-1</sup> (1.45 x 10 <sup>-1</sup> , 2.14 x 10 <sup>-1</sup> )	1.46 x 10 <sup>-1</sup> (1.11 x 10 <sup>-1</sup> , 1.92 x 10 <sup>-1</sup> )	0.66 x 10 <sup>-1</sup> (0.46 x 10 <sup>-1</sup> , 0.94 x 10 <sup>-1</sup> )	**
GA 25	1.14 x 10 <sup>-1</sup> (0.92 x 10 <sup>-1</sup> , 1.41 x 10 <sup>-1</sup> )	1.88 x 10 <sup>-1</sup> (1.39 x 10 <sup>-1</sup> , 2.54 x 10 <sup>-1</sup> )	0.80 x 10 <sup>-1</sup> (0.62 x 10 <sup>-1</sup> , 1.03 x 10 <sup>-1</sup> )	
GA 26	1.21 x 10 <sup>-1</sup> (1.05 x 10 <sup>-1</sup> , 1.39 x 10 <sup>-1</sup> )	1.61 x 10 <sup>-1</sup> (1.38 x 10 <sup>-1</sup> , 1.88 x 10 <sup>-1</sup> )	0.79 x 10 <sup>-1</sup> (0.64 x 10 <sup>-1</sup> , 0.97 x 10 <sup>-1</sup> )	*
GA 27	1.13 x 10 <sup>-1</sup> (1.01 x 10 <sup>-1</sup> , 1.28 x 10 <sup>-1</sup> )	1.45 x 10 <sup>-1</sup> (1.24 x 10 <sup>-1</sup> , 1.70 x 10 <sup>-1</sup> )	0.78 x 10 <sup>-1</sup> (0.63 x 10 <sup>-1</sup> , 0.97 x 10 <sup>-1</sup> )	
<b>B2M / Cr</b>				
GA 24	1.14 x 10 <sup>-2</sup> (0.82 x 10 <sup>-2</sup> , 1.59 x 10 <sup>-2</sup> )	2.57 x 10 <sup>-2</sup> (1.66 x 10 <sup>-2</sup> , 4.00 x 10 <sup>-2</sup> )	2.00 x 10 <sup>-2</sup> (1.22 x 10 <sup>-2</sup> , 3.29 x 10 <sup>-2</sup> )	*
GA 25	0.85 x 10 <sup>-2</sup> (0.61 x 10 <sup>-2</sup> , 1.20 x 10 <sup>-2</sup> )	1.75 x 10 <sup>-2</sup> (1.09 x 10 <sup>-2</sup> , 2.82 x 10 <sup>-2</sup> )	2.83 x 10 <sup>-2</sup> (2.02 x 10 <sup>-2</sup> , 3.97 x 10 <sup>-2</sup> )	**
GA 26	1.52 x 10 <sup>-2</sup> (1.19 x 10 <sup>-2</sup> , 1.94 x 10 <sup>-2</sup> )	1.74 x 10 <sup>-2</sup> (1.31 x 10 <sup>-2</sup> , 2.31 x 10 <sup>-2</sup> )	4.25 x 10 <sup>-2</sup> (3.28 x 10 <sup>-2</sup> , 5.49 x 10 <sup>-2</sup> )	**
GA 27	1.97 x 10 <sup>-2</sup> (1.57 x 10 <sup>-2</sup> , 2.46 x 10 <sup>-2</sup> )	1.69 x 10 <sup>-2</sup> (1.25 x 10 <sup>-2</sup> , 2.29 x 10 <sup>-2</sup> )	2.58 x 10 <sup>-2</sup> (1.76 x 10 <sup>-2</sup> , 3.77 x 10 <sup>-2</sup> )	
<b>Clusterin/Cr</b>				
GA 24	17.3 x 10 <sup>-4</sup> (13.9 x 10 <sup>-4</sup> , 21.4 x 10 <sup>-4</sup> )	9.51 x 10 <sup>-4</sup> (7.34 x 10 <sup>-4</sup> , 12.3 x 10 <sup>-4</sup> )	5.03 x 10 <sup>-4</sup> (3.52 x 10 <sup>-4</sup> , 7.18 x 10 <sup>-4</sup> )	**
GA 25	11.1 x 10 <sup>-4</sup> (9.11 x 10 <sup>-4</sup> , 13.6 x 10 <sup>-4</sup> )	13.8 x 10 <sup>-4</sup> (9.55 x 10 <sup>-4</sup> , 20.0 x 10 <sup>-4</sup> )	4.13 x 10 <sup>-4</sup> (2.84 x 10 <sup>-4</sup> , 6.01 x 10 <sup>-4</sup> )	**
GA 26	8.08 x 10 <sup>-4</sup> (6.93 x 10 <sup>-4</sup> , 9.43 x 10 <sup>-4</sup> )	7.11 x 10 <sup>-4</sup> (5.51 x 10 <sup>-4</sup> , 9.18 x 10 <sup>-4</sup> )	4.00 x 10 <sup>-4</sup> (3.03 x 10 <sup>-4</sup> , 5.28 x 10 <sup>-4</sup> )	**
GA 27	6.30 x 10 <sup>-4</sup> (5.56 x 10 <sup>-4</sup> , 7.14 x 10 <sup>-4</sup> )	6.51 x 10 <sup>-4</sup> (5.27 x 10 <sup>-4</sup> , 8.04 x 10 <sup>-4</sup> )	4.31 x 10 <sup>-4</sup> (3.05 x 10 <sup>-4</sup> , 6.09 x 10 <sup>-4</sup> )	
<b>Cystatin C/Cr</b>				
GA 24	2.28 x 10 <sup>-3</sup> (1.69 x 10 <sup>-3</sup> , 3.09 x 10 <sup>-3</sup> )	2.51 x 10 <sup>-3</sup> (1.76 x 10 <sup>-3</sup> , 3.60 x 10 <sup>-3</sup> )	0.82 x 10 <sup>-3</sup> (0.57 x 10 <sup>-3</sup> , 1.16 x 10 <sup>-3</sup> )	**
GA 25	1.77 x 10 <sup>-3</sup> (1.36 x 10 <sup>-3</sup> , 2.32 x 10 <sup>-3</sup> )	3.12 x 10 <sup>-3</sup> (2.04 x 10 <sup>-3</sup> , 4.79 x 10 <sup>-3</sup> )	0.79 x 10 <sup>-3</sup> (0.56 x 10 <sup>-3</sup> , 1.12 x 10 <sup>-3</sup> )	*
GA 26	1.64 x 10 <sup>-3</sup> (1.33 x 10 <sup>-3</sup> , 2.03 x 10 <sup>-3</sup> )	2.09 x 10 <sup>-3</sup> (1.63 x 10 <sup>-3</sup> , 2.70 x 10 <sup>-3</sup> )	0.98 x 10 <sup>-3</sup> (0.76 x 10 <sup>-3</sup> , 1.26 x 10 <sup>-3</sup> )	*
GA 27	1.27 x 10 <sup>-3</sup> (1.08 x 10 <sup>-3</sup> , 1.49 x 10 <sup>-3</sup> )	1.88 x 10 <sup>-3</sup> (1.51 x 10 <sup>-3</sup> , 2.34 x 10 <sup>-3</sup> )	0.80 x 10 <sup>-3</sup> (0.58 x 10 <sup>-3</sup> , 1.10 x 10 <sup>-3</sup> )	
<b>EGF / Cr</b>				
GA 24	0.85 x 10 <sup>-5</sup> (0.77 x 10 <sup>-5</sup> , 0.93 x 10 <sup>-5</sup> )	1.55 x 10 <sup>-5</sup> (1.41 x 10 <sup>-5</sup> , 1.72 x 10 <sup>-5</sup> )	2.61 x 10 <sup>-5</sup> (2.34 x 10 <sup>-5</sup> , 2.92 x 10 <sup>-5</sup> )	**
GA 25	0.80 x 10 <sup>-5</sup> (0.73 x 10 <sup>-5</sup> , 0.88 x 10 <sup>-5</sup> )	1.37 x 10 <sup>-5</sup> (1.19 x 10 <sup>-5</sup> , 1.58 x 10 <sup>-5</sup> )	2.71 x 10 <sup>-5</sup> (2.46 x 10 <sup>-5</sup> , 2.99 x 10 <sup>-5</sup> )	**
GA 26	0.83 x 10 <sup>-5</sup> (0.78 x 10 <sup>-5</sup> , 0.88 x 10 <sup>-5</sup> )	1.35 x 10 <sup>-5</sup> (1.26 x 10 <sup>-5</sup> , 1.44 x 10 <sup>-5</sup> )	2.77 x 10 <sup>-5</sup> (2.54 x 10 <sup>-5</sup> , 3.02 x 10 <sup>-5</sup> )	**

**Table 3b: Means (95% CI) biomarker/creatinine ratio at specific PMA timepoints by GA groups**

	27 week	30 week	34 week	p-value for trend
GA 27	0.88 x 10 <sup>-5</sup> (0.83 x 10 <sup>-5</sup> , 0.92 x 10 <sup>-5</sup> )	1.23 x 10 <sup>-5</sup> (1.16 x 10 <sup>-5</sup> , 1.31 x 10 <sup>-5</sup> )	2.56 x 10 <sup>-5</sup> (2.35 x 10 <sup>-5</sup> , 2.78 x 10 <sup>-5</sup> )	**
KIM-1 / Cr	**			
GA 24	16.5 x 10 <sup>-7</sup> (13.3 x 10 <sup>-7</sup> , 20.6 x 10 <sup>-7</sup> )	8.62 x 10 <sup>-7</sup> (6.71 x 10 <sup>-7</sup> , 11.1 x 10 <sup>-7</sup> )	7.00 x 10 <sup>-7</sup> (5.62 x 10 <sup>-7</sup> , 8.73 x 10 <sup>-7</sup> )	**
GA 25	12.6 x 10 <sup>-7</sup> (10.4 x 10 <sup>-7</sup> , 15.3 x 10 <sup>-7</sup> )	11.1 x 10 <sup>-7</sup> (8.32 x 10 <sup>-7</sup> , 14.9 x 10 <sup>-7</sup> )	5.74 x 10 <sup>-7</sup> (4.52 x 10 <sup>-7</sup> , 7.29 x 10 <sup>-7</sup> )	**
GA 26	9.20 x 10 <sup>-7</sup> (8.05 x 10 <sup>-7</sup> , 10.5 x 10 <sup>-7</sup> )	9.35 x 10 <sup>-7</sup> (7.79 x 10 <sup>-7</sup> , 11.2 x 10 <sup>-7</sup> )	6.11 x 10 <sup>-7</sup> (4.96 x 10 <sup>-7</sup> , 7.53 x 10 <sup>-7</sup> )	*
GA 27	8.10 x 10 <sup>-7</sup> (7.31 x 10 <sup>-7</sup> , 8.98 x 10 <sup>-7</sup> )	9.95 x 10 <sup>-7</sup> (8.21 x 10 <sup>-7</sup> , 12.1 x 10 <sup>-7</sup> )	7.07 x 10 <sup>-7</sup> (5.52 x 10 <sup>-7</sup> , 9.07 x 10 <sup>-7</sup> )	
NGAL / Cr	**	*		
GA 24	4.67 x 10 <sup>-3</sup> (3.69 x 10 <sup>-3</sup> , 5.92 x 10 <sup>-3</sup> )	1.36 x 10 <sup>-3</sup> (0.97 x 10 <sup>-3</sup> , 1.92 x 10 <sup>-3</sup> )	0.44 x 10 <sup>-3</sup> (0.32 x 10 <sup>-3</sup> , 0.61 x 10 <sup>-3</sup> )	**
GA 25	3.60 x 10 <sup>-3</sup> (2.97 x 10 <sup>-3</sup> , 4.35 x 10 <sup>-3</sup> )	2.05 x 10 <sup>-3</sup> (1.47 x 10 <sup>-3</sup> , 2.87 x 10 <sup>-3</sup> )	0.45 x 10 <sup>-3</sup> (0.34 x 10 <sup>-3</sup> , 0.58 x 10 <sup>-3</sup> )	**
GA 26	2.61 x 10 <sup>-3</sup> (2.24 x 10 <sup>-3</sup> , 3.03 x 10 <sup>-3</sup> )	1.39 x 10 <sup>-3</sup> (1.12 x 10 <sup>-3</sup> , 1.73 x 10 <sup>-3</sup> )	0.36 x 10 <sup>-3</sup> (0.29 x 10 <sup>-3</sup> , 0.44 x 10 <sup>-3</sup> )	**
GA 27	2.30 x 10 <sup>-3</sup> (2.02 x 10 <sup>-3</sup> , 2.61 x 10 <sup>-3</sup> )	1.08 x 10 <sup>-3</sup> (0.90 x 10 <sup>-3</sup> , 1.28 x 10 <sup>-3</sup> )	0.42 x 10 <sup>-3</sup> (0.31 x 10 <sup>-3</sup> , 0.56 x 10 <sup>-3</sup> )	**
OPN / Cr	*	**	*	
GA 24	1.93 x 10 <sup>-3</sup> (1.54 x 10 <sup>-3</sup> , 2.41 x 10 <sup>-3</sup> )	2.20 x 10 <sup>-3</sup> (1.65 x 10 <sup>-3</sup> , 2.92 x 10 <sup>-3</sup> )	2.29 x 10 <sup>-3</sup> (1.68 x 10 <sup>-3</sup> , 3.11 x 10 <sup>-3</sup> )	
GA 25	1.55 x 10 <sup>-3</sup> (1.26 x 10 <sup>-3</sup> , 1.90 x 10 <sup>-3</sup> )	1.59 x 10 <sup>-3</sup> (1.08 x 10 <sup>-3</sup> , 2.35 x 10 <sup>-3</sup> )	1.53 x 10 <sup>-3</sup> (1.05 x 10 <sup>-3</sup> , 2.22 x 10 <sup>-3</sup> )	
GA 26	2.18 x 10 <sup>-3</sup> (1.92 x 10 <sup>-3</sup> , 2.46 x 10 <sup>-3</sup> )	1.09 x 10 <sup>-3</sup> (0.86 x 10 <sup>-3</sup> , 1.38 x 10 <sup>-3</sup> )	1.70 x 10 <sup>-3</sup> (1.35 x 10 <sup>-3</sup> , 2.15 x 10 <sup>-3</sup> )	*
GA 27	2.39 x 10 <sup>-3</sup> (2.15 x 10 <sup>-3</sup> , 2.67 x 10 <sup>-3</sup> )	1.05 x 10 <sup>-3</sup> (0.85 x 10 <sup>-3</sup> , 1.30 x 10 <sup>-3</sup> )	1.09 x 10 <sup>-3</sup> (0.77 x 10 <sup>-3</sup> , 1.55 x 10 <sup>-3</sup> )	**
UMOD / Cr	*			
GA 24	0.77 x 10 <sup>-2</sup> (0.66 x 10 <sup>-2</sup> , 0.89 x 10 <sup>-2</sup> )	1.18 x 10 <sup>-2</sup> (1.00 x 10 <sup>-2</sup> , 1.38 x 10 <sup>-2</sup> )	1.57 x 10 <sup>-2</sup> (1.31 x 10 <sup>-2</sup> , 1.88 x 10 <sup>-2</sup> )	**
GA 25	0.63 x 10 <sup>-2</sup> (0.56 x 10 <sup>-2</sup> , 0.71 x 10 <sup>-2</sup> )	1.09 x 10 <sup>-2</sup> (0.89 x 10 <sup>-2</sup> , 1.32 x 10 <sup>-2</sup> )	1.72 x 10 <sup>-2</sup> (1.42 x 10 <sup>-2</sup> , 2.08 x 10 <sup>-2</sup> )	**
GA 26	0.72 x 10 <sup>-2</sup> (0.66 x 10 <sup>-2</sup> , 0.78 x 10 <sup>-2</sup> )	1.07 x 10 <sup>-2</sup> (0.97 x 10 <sup>-2</sup> , 1.19 x 10 <sup>-2</sup> )	1.81 x 10 <sup>-2</sup> (1.59 x 10 <sup>-2</sup> , 2.06 x 10 <sup>-2</sup> )	**
GA 27	0.85 x 10 <sup>-2</sup> (0.78 x 10 <sup>-2</sup> , 0.91 x 10 <sup>-2</sup> )	1.10 x 10 <sup>-2</sup> (1.00 x 10 <sup>-2</sup> , 1.21 x 10 <sup>-2</sup> )	1.75 x 10 <sup>-2</sup> (1.49 x 10 <sup>-2</sup> , 2.05 x 10 <sup>-2</sup> )	**

Urine Biomarker Concentrations Reported as pg/ml; urine biomarker/creatinine ratios reported as unitless term

\*\* Test for trend across time point significant at p < 0.001

\* Test for trend across time point significant at p < 0.05

**Table 4:** Predicted mean (SD) biomarker and biomarker/cr at birth and slope for % change per week by gender

Biomarker (pg/ml)		Biomarker/Cr (unitless)	
	Birth fitted value	change per week	
<b>αGST</b>	**		
Male	13.5 (11.6, 15.8)	-13.4 (-18.1, -8.49)	αGST / cr
Female	19.8 (16.8, 23.3)	-10.4 (-15.7, -4.91)	Birth fitted value
Albumin		*	
Male	1.09 x 10 <sup>7</sup> (1.00 x 10 <sup>7</sup> , 1.18 x 10 <sup>7</sup> )	6.76 (3.51, 10.1)	
Female	1.13 x 10 <sup>7</sup> (1.04 x 10 <sup>7</sup> , 1.22 x 10 <sup>7</sup> )	13.5 (10.2, 17.0)	
B2M	*	*	
Male	1.45 x 10 <sup>6</sup> (1.27 x 10 <sup>6</sup> , 1.67 x 10 <sup>6</sup> )	3.39 (-1.61, 8.65)	B2M / cr
Female	1.83 x 10 <sup>6</sup> (1.61 x 10 <sup>6</sup> , 2.09 x 10 <sup>6</sup> )	-4.16 (-8.83, 0.76)	
Clusterin	*		
Male	6.75 x 10 <sup>4</sup> (6.14 x 10 <sup>4</sup> , 7.42 x 10 <sup>4</sup> )	11.5 (7.25, 15.9)	
Female	7.74 x 10 <sup>4</sup> (7.02 x 10 <sup>4</sup> , 8.54 x 10 <sup>4</sup> )	5.99 (1.74, 10.4)	
Cystatin C	**	*	
Male	1.55 x 10 <sup>5</sup> (1.38 x 10 <sup>5</sup> , 1.75 x 10 <sup>5</sup> )	9.98 (5.19, 15.0)	Cystatin C / cr
Female	2.10 x 10 <sup>5</sup> (1.86 x 10 <sup>5</sup> , 2.38 x 10 <sup>5</sup> )	-0.59 (-5.04, 4.07)	
EGF			
Male	5.35 x 10 <sup>2</sup> (5.09 x 10 <sup>2</sup> , 5.62 x 10 <sup>2</sup> )	26.1 (23.8, 28.5)	
Female	5.70 x 10 <sup>2</sup> (5.43 x 10 <sup>2</sup> , 5.97 x 10 <sup>2</sup> )	24.7 (22.3, 27.1)	
KIM-1	*		
Male	76.5 (70.6, 82.9)	5.88 (2.67, 9.20)	KIM-1 / cr
Female	91.5 (84.1, 99.5)	8.21 (4.69, 11.9)	
NGAL	**		
Male	2.21 x 10 <sup>5</sup> (1.99 x 10 <sup>5</sup> , 2.45 x 10 <sup>5</sup> )	-12.9 (-16.4, -9.39)	
Female	3.67 x 10 <sup>5</sup> (3.36 x 10 <sup>5</sup> , 4.01 x 10 <sup>5</sup> )	-11.8 (-14.9, -8.65)	
OPN		*	

Biomarker (pg/ml)		Biomarker/Cr (unitless)	
	Birth fitted value	change per week	
Male	1.94 x 10 <sup>5</sup> (1.79 x 10 <sup>5</sup> , 2.10 x 10 <sup>5</sup> )	-5.95 (-9.22, -2.57)	2.69 x 10 <sup>-3</sup> (2.47 x 10 <sup>-3</sup> , 2.92 x 10 <sup>-3</sup> )
Female	2.02 x 10 <sup>5</sup> (1.85 x 10 <sup>5</sup> , 2.21 x 10 <sup>5</sup> )	-12.4 (-15.9, -8.79)	2.89 x 10 <sup>-3</sup> (2.63 x 10 <sup>-3</sup> , 3.17 x 10 <sup>-3</sup> )
UMOD			UMOD / Cr
Male	4.96 x 10 <sup>5</sup> (4.69 x 10 <sup>5</sup> , 5.24 x 10 <sup>5</sup> )	21.3 (18.9, 23.7)	6.88 x 10 <sup>-3</sup> (6.53 x 10 <sup>-3</sup> , 7.26 x 10 <sup>-3</sup> )
Female	5.18 x 10 <sup>5</sup> (4.91 x 10 <sup>5</sup> , 5.46 x 10 <sup>5</sup> )	21.3 (18.8, 23.7)	7.39 x 10 <sup>-3</sup> (7.01 x 10 <sup>-3</sup> , 7.79 x 10 <sup>-3</sup> )
Creatinine			
Male	7.21 (6.91, 7.51)	10.6 (8.89, 12.3)	
Female	**		

\*\* differences across GA are significant at p < 0.001

\* differences across GA are significant at p < 0.05

**Table 5:**

Predicted mean (SD) biomarker at birth and slope for % change per week by Epo status

	Biomarker (pg/ml)	
	Birth fitted value	change per week
aGST		
Epo	17.0 (14.5, 19.9)	-13.6 (-18.5, -8.42)
Placebo	15.6 (13.4, 18.3)	-9.95 (-15.0, -4.61)
Albumin		
Epo	$1.13 \times 10^7$ ( $1.04 \times 10^7$ , $1.23 \times 10^7$ )	8.30 (4.90, 11.8)
Placebo	$1.09 \times 10^7$ ( $1.01 \times 10^7$ , $1.18 \times 10^7$ )	12.0 (8.74, 15.4)
B2M		
Epo	$1.71 \times 10^6$ ( $1.50 \times 10^6$ , $1.95 \times 10^6$ )	-1.72 (-6.54, 3.35)
Placebo	$1.55 \times 10^6$ ( $1.36 \times 10^6$ , $1.78 \times 10^6$ )	0.83 (-4.03, 5.93)
Clusterin		
Epo	$7.47 \times 10^4$ ( $6.76 \times 10^4$ , $8.26 \times 10^4$ )	7.25 (2.82, 11.9)
Placebo	$6.98 \times 10^4$ ( $6.36 \times 10^4$ , $7.66 \times 10^4$ )	10.1 (6.04, 14.3)
Cystatin C		
Epo	$1.84 \times 10^5$ ( $1.63 \times 10^5$ , $2.09 \times 10^5$ )	3.22 (-1.57, 8.25)
Placebo	$1.76 \times 10^5$ ( $1.56 \times 10^5$ , $1.99 \times 10^5$ )	5.88 (1.41, 10.5)
EGF		
Epo	$5.67 \times 10^2$ ( $5.40 \times 10^2$ , $5.96 \times 10^2$ )	24.4 (22.0, 26.9)
Placebo	$5.37 \times 10^2$ ( $5.11 \times 10^2$ , $5.63 \times 10^2$ )	26.4 (24.2, 28.7)
KIM-1		
Epo	85.2 (78.5, 92.4)	5.19 (1.92, 8.55)
Placebo	81.9 (75.4, 89.0)	9.04 (5.56, 12.6)
NGAL		
Epo	$2.77 \times 10^5$ ( $2.50 \times 10^5$ , $3.06 \times 10^5$ )	-12.1 (-15.5, -8.52)
Placebo	$2.90 \times 10^5$ ( $2.64 \times 10^5$ , $3.19 \times 10^5$ )	-12.3 (-15.5, -8.97)
OPN		
Epo	$2.05 \times 10^5$ ( $1.88 \times 10^5$ , $2.23 \times 10^5$ )	-9.94 (-13.3, -6.48)
Placebo	$1.91 \times 10^5$ ( $1.75 \times 10^5$ , $2.08 \times 10^5$ )	-8.66 (-12.1, -5.08)
UMOD		
Epo	$5.24 \times 10^5$ ( $4.95 \times 10^5$ , $5.54 \times 10^5$ )	21.5 (18.9, 24.1)
Placebo	$4.89 \times 10^5$ ( $4.64 \times 10^5$ , $5.16 \times 10^5$ )	21.2 (19.0, 23.5)
Creatinine		
Epo	7.21 (6.93, 7.50)	10.0 (8.34, 11.8)
Placebo	7.01 (6.73, 7.31)	11.4 (9.68, 13.1)

\*\* differences across GA are significant at  $p < 0.001$

\* differences across GA are significant at  $p < 0.05$

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

**Table 6:**

Summary of biomarker changes from time of birth, and time of conception

	$\alpha$ GST	Albumin	B2M	Clusterin	Creatinine	Cystatin C	EGF	KIM-1	NGAL	OPN	UMOD
Different by GA at birth	**	**	**	**	**	**	**	**	**	**	**
Different rate of change from birth by GA	**	**	*			**				*	
Different value at 27 week PMA by GA		**	*	**	*	*		**	**		
Different value at 30 week PMA by GA				*			*	**	*	**	
Different value at 34 week PMA by GA										*	
Different over time from conception 24 week GA		**	*	**		**	**	**	**		**
Different over time from conception 25 week GA			**	*	**		**	*	**		**
Different over time from conception 26 week GA			**	*	**		**		**		**
Different over time from conception 27 week GA			*		**		**	*	**	**	**
Different by sex at birth	**		*	*		**		**	**		**
Different rate of change from birth by sex		*	*			*				*	
Different by treatment status (EPO vs. Placebo)											

\*\* differences across GA are significant at  $p < 0.001$

\* differences across GA are significant at  $p < 0.05$