Assessment of renal functional maturation and injury in preterm neonates during the first month of life

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1Preventative Health, Baker IDI Heart and Diabetes Institute, Melbourne, Victoria, Australia; 2Department of Anatomy and Developmental Biology, Monash University, Clayton, Victoria, Australia; 3Richie Centre for Baby Health Research, Monash Institute of Medical Research, Clayton, Victoria, Australia; 4Monash Newborn, Monash Medical Centre, Clayton, Victoria, Australia; 5Department of Neonatology, Canberra Hospital, and the Australian National University Medical School, Canberra, Australian Capital Territory, Australia; 6Department of Surgical Pathology, South Australia Pathology, Women’s and Children’s Hospital, North Adelaide and the University of Adelaide, Adelaide, South Australia, Australia; 7Menzies School of Health Research and the Royal Darwin Hospital, Casuarina, Northern Territory, Australia; and 8Centre for Chronic Disease, University of Queensland, Brisbane, Queensland, Australia

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Gubhaju L, Sutherland MR, Horne RS, Medhurst A, Kent AL, Ramsden A, Moore L, Singh G, Hoy WE, Black MJ. Assessment of renal functional maturation and injury in preterm neonates during the first month of life. Am J Physiol Renal Physiol 307: F149–F158, 2014. First published June 4, 2014; doi:10.1152/ajprenal.00439.2013.—Worldwide, approximately 10% of neonates are born preterm. The majority of preterm neonates are born when the kidneys are still developing; therefore, during the early postnatal period renal function is likely reflective of renal immaturity and/or injury. This study evaluated glomerular and tubular function and urinary neutrophil gelatinase-associated lipocalin (NGAL; a marker of renal injury) in preterm neonates during the first month of life. Preterm and term infants were recruited from Monash Newborn (neonatal intensive care unit at Monash Medical Centre) and Jesse McPherson Private Hospital, respectively. Infants were grouped according to gestational age at birth: ≤28 wk (n = 33), 29–31 wk (n = 44), 32–36 wk (n = 32), and term (≥37 wk (n = 22)). Measures of glomerular and tubular function were assessed on postnatal days 3–7, 14, 21, and 28. Glomerular and tubular function was significantly affected by gestational age at birth, as well as by postnatal age. By postnatal day 28, creatinine clearance remained significantly lower among preterm neonates compared with term infants; however, sodium excretion was not significantly different. Pathological proteinuria and high urinary NGAL levels were observed in a number of neonates, which may be indicative of renal injury; however, there was no correlation between the two markers. Findings suggest that neonatal renal function is predominantly influenced by renal maturity, and there was high capacity for postnatal tubular maturation among preterm neonates. There is insufficient evidence to suggest that urinary NGAL is a useful marker of renal injury in the preterm neonate. 

Renal function in the preterm neonate is affected by renal immaturity and potential injury during the early postnatal period. At the time when the majority of preterm infants are born, renal development is still ongoing (48) and renal function is accordingly immature (15). Preterm neonates have been shown to have a low glomerular filtration rate (GFR) compared with term neonates, and the tubules excrete high amounts of sodium (2). Furthermore, compared with babies born at term, preterm neonates may demonstrate a slower progression in renal functional maturation after birth (5, 14). Creatinine clearance (CCr) has been shown to be positively correlated with both gestational age and postnatal age (2, 5, 7–9, 12, 14, 21, 39, 47, 51, 56), while the fractional excretion of sodium (FE\textsubscript{Na}) has been shown to be inversely correlated with gestational age (12) and postnatal age (2, 12, 13, 39, 47).

Only a few studies to date have investigated the occurrence of proteinuria following preterm birth. Very low concentrations of both high-molecular-weight (HMW) and low-molecular-weight (LMW) proteins are normally present in the urine, due to the function of the glomerular filtration barrier and the reuptake of filtered proteins in the proximal tubule (28, 32). A limited number of previous studies have demonstrated a high variation in urine albumin levels between individual preterm neonates (7, 10), with the highest levels exhibited by those with a low gestational age at birth and those that are clinically unstable (3, 7, 10, 11, 52). Urinary levels of β2-microglobulin (β2-M) have also been shown to be significantly greater in the preterm infant compared with term-born infants throughout the first month of life (2, 52, 53), and they decrease with increasing gestational and postnatal age (50). Proteinuria is known to be an important indicator of renal injury; however, to date it remains unclear whether the increased urinary protein levels reported in preterm neonates are associated with renal immaturity and/or acute renal injury.

The preterm kidney is highly susceptible to injury in the neonatal period; acute kidney injury (AKI) is reported to occur in 8–24% of preterm neonates admitted to the neonatal intensive care unit (19, 44) and is primarily prerenal in origin (6, 44). In a large study of preterm infants born in the United States and Puerto Rico, Walker et al. (55) examined the medical records of 66,526 neonates (born at ≤30-wk gestation); 4% of the neonates were diagnosed with renal dysfunction and/or renal failure. The predominant risk factors for impaired renal function were low gestational age and low birth weight. Further risk factors included postnatal medication administration (vasopressors, indomethacin, and antibiotics), postnatal illness (intraventricular hemorrhage, a patent ductus arteriosus, necrotising enterocolitis, culture positive sepsis), and also the use of high-frequency ventilation, male gender,

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and non-white race. Importantly, mortality rates were significantly higher in neonates with the diagnosis of renal dysfunction and/or renal failure (55). In addition, renal injury in the preterm neonate may be an antecedent to chronic renal disease (1). Therefore, the early diagnosis of AKI is paramount so that conservative management of AKI can be initiated in time to potentially prevent these long-term consequences. In this regard, urinary neutrophil gelatinase-associated lipocalin (NGAL) has recently been investigated as a potential biomarker of AKI in preterm neonates, with studies showing that NGAL levels strongly correlated with both gestational and postnatal age (20, 23) and are highest in neonates that are critically ill (23, 33).

In this Australian-based study, we have examined renal function in preterm infants admitted to the neonatal intensive care unit at the Monash Medical Centre (a large tertiary level hospital, located in Melbourne, Australia); the current rate of preterm birth in the Australian population is 8.2% (24). The aims of the study were to (1) assess postnatal renal function from day 3 to day 28 in extremely preterm (≤28 wk of gestation), very preterm (29–31 wk of gestation), and moderately preterm (32–36 wk of gestation) neonates compared with term controls (37–42 wk of gestation) by examining glomerular (CCr, and urine albumin) and tubular (FENa, and urine β2-M) function; and (2) to determine whether urinary NGAL is a useful marker of renal injury in preterm neonates.

MATERIALS AND METHODS

Ethics Statement

Ethics approval for this study was obtained from the Southern Health Human Research Ethics Committee and the Monash University Standing Committee on Ethics in Research Involving Humans. Written informed parental consent was obtained for all participants in the study.

Study Population

Preterm neonates (<37 wk of gestation) admitted to the neonatal intensive care unit at Monash Medical Centre and term infants (37–42 wk of gestation) born at Jessie MacPherson Private Hospital (Clayton, Victoria, Australia) without any congenital abnormalities, were eligible for the study. Between April 2008 and October 2011, 143 neonates were recruited into the study. Four neonates were excluded following the early withdrawal of parental consent. Eight preterm neonates were further excluded following transfer to other hospitals before postnatal day 7. Two extremely preterm neonates died before the study was completed. The remaining 129 neonates were stratified into four groups according to gestational age: group A (≤28 wk of gestation; n = 33); group B (29–31 wk of gestation; n = 44); group C (32–36 wk of gestation; n = 30); and group D (≥37 wk of gestation; n = 22).

Urine Collection Procedure

Sanitary pads (Kotex; Kimberly-Clark, NSW, Australia) were placed within diapers to collect urine samples, a method that has been previously validated (18, 45). A diaper liner (Johnson’s Baby Nappy Liners; Johnson&Johnson Pacific, NSW, Australia) was also placed inside the diaper to filter out any feces. To estimate urine flow rate, all diapers inclusive of the pad and liner were weighed before and after use, and the time each diaper was put on the baby was recorded (38). In the case of missed voids or a heavily soiled diaper, a value of average urine output for the relevant time period (calculated from all other diapers within that 24-h period) was substituted for the missing value. Diapers were changed at the discretion of nursing staff and/or parents (ranging from every 8 h in the extremely preterm neonates to <2 h in the term neonates), and collected in a sealed plastic container. At least twice per day, diapers were collected from the nursery and the urine was extracted by compressing the sanitary pad using a hydraulic press (18, 45). All urine collected from the diapers over a 24-h period was pooled before analysis.

It has been shown that urine collected from disposable cotton pads and/or cotton wool does not affect the urinary constituent of sodium, potassium, or creatinine (38) and has been used previously in the analysis of urinary NGAL (23). Previous research has shown that protein can get bound within cotton material (43). Therefore, spot urine samples collected using urine collection bags were utilized to determine urine total protein, albumin, and β2-microglobulin levels.

In preterm neonates, 24-h urine collection from diapers began at 72 h after birth (day 3) and continued until postnatal day 7. In addition, 24-h urine was collected on days 14, 21, and 28 of life. Spot urine samples (1–2 ml) were obtained on days 7, 14, 21, and 28 of life. In term neonates, 24-h urine collection commenced 48 h after birth (day 2) and continued until the infant was discharged from the hospital (~day 4 of life). Additionally, urine was collected for a 24-h period on day 28 of life. For those infants who had been discharged, diapers were delivered and collected from the infant’s home (group A, n = 0; group B, n = 0; group C, n = 7; and group D, n = 11). Spot urine samples were obtained from the term neonates on day 3 of life. Pooled 24-h urine samples were frozen at −20°C until analysis. Spot urine samples were sent for analysis of urinary protein levels immediately after collection.

For a number of the infants, the urine collections and analyses on days 14–28 were not performed, primarily due to the transfer of infants to private hospitals, or discharge from the hospital; group A [n = 1/33 (3.0%)]; group B [n = 8/44 (18.2%)]; group C [n = 11/30 (33.3%)]; and group D [n = 11/22 (50.0%)].

Assessment of Renal Function

Urinary and plasma sodium and creatinine. All urine analyses were performed by the Southern Health Pathology Department (Southern Cross Pathology; Clayton, Victoria, Australia). Pooled 24-h urine samples were analyzed for sodium and creatinine levels. Plasma creatinine and plasma sodium levels were recorded from blood tests that were undertaken as part of the routine care of preterm neonates, and data were extracted from the medical records. In term neonates, heelpich blood samples were obtained for analysis at the time of the routine newborn screening test (at ~48 h of life). In cases where a blood test was not available in term infants on postnatal day 28 (group D, n = 11), average levels of plasma sodium (140 mmol/l) and plasma creatinine (40 μmol/l) that are within the expected range for term infants were used in the calculation of CCr and FENa.

Calculation of CCr and FENa. The estimation of GFR using CCr is generally considered to be a more accurate measure of glomerular function than serum creatinine alone. Although it is to be noted that the potential for active tubular secretion of creatinine, as well as differences in muscle mass between neonates, may affect the accuracy of the results (46). CCr was calculated using the following formula:

\[
CCr = \frac{UCr \times PCr}{(URC \times PCr) \times BSA}
\]

where UCr = urinary creatinine (μmol/l); urine flow rate = ml/min (calculated for each 24-h period); PCr = plasma creatinine (μmol/l); and BSA = body surface area (m²).

BSA was calculated using the following formula derived by Haycock et al. (17):

\[
BSA = \left(\frac{3964}{0.5378 \times b \times 0.3964}\right) \times \left(\frac{w}{0.024265}\right)^{0.5378}
\]

FENa was calculated using the following formula:
RENAL FUNCTION AND INJURY IN PRETERM NEONATES

FE_{Na}(\%) = (UNa/PNa) * (PCr/Ucr) * 100,

where UNa = urinary sodium (mmol/l); PNa = plasma sodium (mmol/l); PCr = plasma creatinine (\mu mol/l); and UCr = urinary creatinine (\mu mol/l).

Urinary total protein, albumin, and \beta-M. Urine total protein (UTP), urinary albumin, and urinary \beta-M were measured in spot urine samples using nephelometric technology on a Beckman immunochemistry system, with reagents and calibrators supplied by Beckman Diagnostics (urine total protein and urine albumin; Beckman Diagnostics; Sydney, Australia) and DakoCytomation (\beta-M; DakoCytomation; Glostrup, Denmark). In instances where UTP was greater than 500 mg/l, this was defined as pathological proteinuria and urine albumin levels were not determined. All urine protein levels were expressed as a ratio to urine creatinine concentrations.

It is to be noted that missing data exist for the urine protein results due to difficulties in obtaining clean spot urine samples from a number of the infants (urine collection bags could not be placed on some extremely preterm neonates due to their delicate skin and/or parents not providing consent). Overall, 46.2% of the total number of requested spot urine samples were obtained. At least one spot urine sample was obtained for the majority of neonates: group A (91%), group B (93%), group C (87%), and group D (27%).

Urinary NGAL. In a subset of the study participants (61.2%), pooled 24-h urine samples from one or more postnatal time points were analyzed for urinary NGAL levels. Urinary NGAL analysis was performed using a sandwich ELISA in microwells coated with a monoclonal antibody against human NGAL (NGAL ELISA Kit, BioPorto Diagnostics; Gentofte, Denmark); the upper limit of the test was 500 ng/ml. In accordance with previous studies, urinary NGAL levels were expressed as nanograms per milliliter and were not corrected for urine creatinine concentrations (20, 23, 33).

Impaired renal function. Impaired renal function (low urine output, low creatinine clearance, high serum creatinine, high fractional excretion of sodium, high urine total protein and high urinary NGAL) was defined as values that differed more than 2 SD from the mean at any time point from postnatal day 3 through to postnatal day 28. The mean and SD was calculated from either absolute values (SCr) or plasma creatinine (\mu mol/l); and UCr = urinary creatinine (\mu mol/l).

RESULTS

Statistical Analysis

Statistical analyses were performed using GraphPad Prism v5.04 for Windows and Intercooled Stata v8.0 for Windows. Data are presented as the means ± SE. Statistical significance was accepted at the level of \( P < 0.05 \).

Birth characteristics (gestational age, birth weight, length, head circumference) were compared among groups using a one-way ANOVA, followed by a Bonferroni post hoc test. To determine differences in categorical variables among groups (such as sex, disease outcomes, and medication administration), a Fisher’s exact test was performed.

Urinary output, C_GFR, and FE_{Scr} in preterm neonates (groups A, B, and C) were analyzed using a two-way ANOVA with repeated measures to assess renal functional maturation with increasing postnatal age. At postnatal days 3 and 28, urine output, C_GFR, and the FE_{Scr} (in all 4 groups) were analyzed using a two-way ANOVA, followed by a Bonferroni post hoc test to determine differences between individual groups at each time point; this analysis enabled comparison between preterm and term infants at the start and end points of the study. Urine protein (UTP, albumin, and \beta-M)-to-creatinine ratios and urine NGAL levels were also analyzed using a two-way ANOVA, followed by a Bonferroni post hoc test. The factors assessed in all of these analyses were gestational age (pGA), postnatal age (pPA), and their interaction (pGA × pPA).

Linear regression analyses [followed by an analysis of covariance (ANCOVA)] were used to compare the rate of change in C_GFR and FE_{Scr} from postnatal day 3 to day 28, and also to determine whether urinary NGAL levels correlated with any other indices of renal function. Additionally, urinary NGAL levels in neonates exhibiting pathological proteinuria (UTP ≥ 500 mg/l) were compared with age- and sex-matched neonates (controls) with lower urine total protein levels (UTP < 480 mg/l, and less than half of the UTP level of the matched neonate with pathological proteinuria) at the corresponding postnatal time point. This analysis was undertaken using a two-way ANOVA with the factors proteinuria (pP), gestational age (pGA), and their interaction (pP × pGA). Additionally, in these two groups, urinary NGAL levels were assessed at time points before (7 days if pathological proteinuria observed at day 14, 21, or 28; 3 days if observed at day 7) and at the time of proteinuria onset. This analysis was undertaken using a two-way ANOVA with the factors proteinuria (pP), time point of assessment (pT), and their interaction (pP × pT).

Pregnancy and Neonatal Birth Characteristics

Reasons for preterm delivery included onset of spontaneous preterm labor (34.0%), premature prelabour rupture of membranes (30.1%), placenta previa/apruption (15.5%), and clinical indication due to maternal and/or fetal health risks such as preeclampsia and suspected fetal compromise (20.4%). There were a significantly higher proportion of births attributed to spontaneous preterm labor in group A (extremely preterm) neonates compared with both group B and group C neonates. The majority of preterm neonates (> 65%) were born via caesarean delivery, compared with 27% among term infants (Table 1). At least 70% of the mothers of preterm neonates received antenatal steroids before delivery; 94% of those born extremely preterm (group A) received antenatal steroids.

Body weight, length, and head circumference at birth all increased significantly along with increasing gestational age (Table 1). There was no significant difference in the gender balance between gestational age groups. Groups A and C had the greatest number of small-for-gestational age (SGA) neonates; there were a similar number of multiple births in each of the preterm groups whereas all term-born neonates in group D were singletons. Of the preterm groups, there was no significant difference in birth weight between the singletons and multiples (group A: singleton 818.6 ± 36.8 g, multiple 790.9 ± 35.8 g; group B: singleton 1,429 ± 47.9 g, multiple 1,468 ± 65.0 g; group C: singleton 1,767 ± 111.4 g, multiple 1,778 ± 93.4 g). The majority of neonates that had a low Apgar score (> 7) at 5 min were in group A (Table 2).

Postnatal Neonatal Complications and Medications Administered

Since the majority of preterm neonates required mechanical ventilation after birth, respiratory distress syndrome was very common in these groups (Table 2). The occurrence of culture-positive sepsis and a patent ductus arteriosus was significantly greater in group A compared with those infants in older gestational age groups. Only neonates within group A and group D were diagnosed with intraventricular hemorrhage.
Table 1. Pregnancy and birth characteristics of neonates by gestational age group

<table>
<thead>
<tr>
<th></th>
<th>Group A, ≤28 wk (n = 33)</th>
<th>Group B, 29–31 wk (n = 44)</th>
<th>Group C, 32–36 wk (n = 30)</th>
<th>Group D, ≥37 wk (n = 22)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age, wk</td>
<td>26.6 ± 0.2* (24–28)</td>
<td>30.4 ± 0.1* (29–31)</td>
<td>33.7 ± 0.2* (32–36)</td>
<td>39.6 ± 0.2* (37–42)</td>
</tr>
<tr>
<td>Birth weight, g</td>
<td>811.1 ± 28.3* (529–1,229)</td>
<td>1,450.0 ± 39.6* (892–2,157)</td>
<td>1,771.0 ± 75.4* (1,018–2,542)</td>
<td>3,356.0 ± 76.2* (2,820–4,160)</td>
</tr>
<tr>
<td>Body length, cm</td>
<td>33.6 ± 0.4* (29–39)</td>
<td>40.9 ± 0.4* (36–46)</td>
<td>43.4 ± 0.7* (36–50)</td>
<td>49.9 ± 0.4* (46–55)</td>
</tr>
<tr>
<td>Head circumference, cm</td>
<td>23.9 ± 0.2* (21–27)</td>
<td>28.3 ± 0.2* (25–32)</td>
<td>29.9 ± 0.4* (26–33)</td>
<td>34.0 ± 0.3* (32–36)</td>
</tr>
<tr>
<td>Male, %</td>
<td>36.4</td>
<td>52.3</td>
<td>46.7</td>
<td>50.0</td>
</tr>
<tr>
<td>SGA, %</td>
<td>30.3‡§ 9.1‡¶</td>
<td>46.7‡§ 4.5‡¶</td>
<td>80.8†‡ 0.0¶</td>
<td>0.0*</td>
</tr>
<tr>
<td>Twin/triplet, %</td>
<td>27.3†</td>
<td>94.7‡</td>
<td>80.8†</td>
<td>0.0*</td>
</tr>
<tr>
<td>Antenatal steroids</td>
<td>3.1*</td>
<td>12.5</td>
<td>3.1*</td>
<td>0.0*</td>
</tr>
<tr>
<td>Caesarean</td>
<td>3.1*</td>
<td>68.8*</td>
<td>64.3*</td>
<td>26.1*</td>
</tr>
</tbody>
</table>

Values are means ± SE (range) and as noted. SGA, small for gestational age. Significant differences (P < 0.05) between groups are indicated by symbols: *vs. all other groups; †vs. group D; ‡vs. group C; §vs. group B; ¶vs. group A.

Among preterm neonates, the most commonly administered medications were a routine regimen of antibiotics. The majority of neonates that received additional antibiotics were in the extremely preterm group (group A). In general, the administration of drugs was significantly greater among neonates in group A. Term infants (group D) did not receive any medications over the course of the study.

Renal Function

Urinary output. Neither gestational age at birth nor postnatal age affected urine output in preterm neonates from postnatal day 3 to day 7 (Fig. 1A). On postnatal days 14–28, however, urine output was significantly greater in neonates with increased gestational age at birth (Fig. 1B). Urine output was significantly lower in term neonates compared with preterm neonates at postnatal day 3; however, there was no difference between groups at postnatal day 28 (Fig. 1C). Oliguria (urine output <1 ml·kg⁻¹·h⁻¹) was observed in 10 term infants (postnatal day 3) and 1 preterm neonate (postnatal day 5).

Ccr. During the first week and month of life, Ccr was positively associated with both gestational age at birth and postnatal age (Fig. 1, D and E). Of the preterm neonates, groups B and C had the highest Ccr throughout the study period. On postnatal day 28, Ccr levels were not significantly different among the preterm groups; however, term neonates had significantly higher Ccr compared with preterm neonates (Fig. 1F). Linear regression analyses showed that there was no significant difference in the rate of change in Ccr from postnatal day 3 to postnatal day 28 among all groups of neonates (data not shown).

FENa. FENa was inversely associated with gestational age at birth during the first week and month of life (Fig. 1, G and H). The highest levels of sodium excretion were observed in group A throughout the study period. On postnatal day 3 (Fig. 1I), FENa was significantly lower in term neonates (group D) than in all other groups. By postnatal day 28, however, there was no significant difference in FENa among groups. Linear regression analysis showed that the rate of change in FENa from postnatal day 3 to day 28 was significantly greater in group B neonates compared with group D (P < 0.0001) and was also significantly greater in group A neonates compared with all other groups (P ≤ 0.02; data not shown).

UPT, albumin, and β-M. UTP, albumin, and β-M levels in preterm neonates (corrected for urine creatinine) were all

Table 2. Percentage of neonates with postnatal complications and percentage exposed to medications during the early postnatal period in each gestational age group

<table>
<thead>
<tr>
<th>Postnatal complications</th>
<th>Group A, ≤28 wk (n = 33) (%)</th>
<th>Group B, 29–31 wk (n = 44) (%)</th>
<th>Group C, 32–36 wk (n = 30) (%)</th>
<th>Group D, ≥37 wk (n = 22) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mechanical ventilation</td>
<td>45.5*</td>
<td>25.6*</td>
<td>3.3‡¶</td>
<td>0.0‡¶</td>
</tr>
<tr>
<td>Respiratory distress syndrome</td>
<td>100‡</td>
<td>84.1‡</td>
<td>56.7*</td>
<td>0.0*</td>
</tr>
<tr>
<td>Culture positive sepsis</td>
<td>50.0*</td>
<td>12.2†</td>
<td>3.3†</td>
<td>0.0†</td>
</tr>
<tr>
<td>Intraventricular hemorrhage</td>
<td>31.3†</td>
<td>14.6</td>
<td>0.0†</td>
<td>0.0†</td>
</tr>
<tr>
<td>Patent ductus arteriosus</td>
<td>68.8*</td>
<td>15.0*</td>
<td>3.3†</td>
<td>0.0†</td>
</tr>
<tr>
<td>Antibiotics</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-Lactam (benzylpenicillin, imipenem, ampicillin)</td>
<td>100‡</td>
<td>100‡</td>
<td>80.0*</td>
<td>0.0*</td>
</tr>
<tr>
<td>Aminoglycoside (gentamicin)</td>
<td>100‡</td>
<td>100‡</td>
<td>80.0*</td>
<td>0.0*</td>
</tr>
<tr>
<td>Glycopeptide (vancomycin)</td>
<td>57.6*</td>
<td>16.7†</td>
<td>10.0†</td>
<td>0.0†</td>
</tr>
<tr>
<td>Macrolide (erythromycin)</td>
<td>12.5</td>
<td>2.4</td>
<td>3.3</td>
<td>0.0</td>
</tr>
<tr>
<td>Cephalosporin (cefotaxime, cefozolin)</td>
<td>12.5</td>
<td>2.4</td>
<td>3.3</td>
<td>0.0</td>
</tr>
<tr>
<td>Nitroimidazole (metronidazole)</td>
<td>3.1</td>
<td>0.0</td>
<td>3.3</td>
<td>0.0</td>
</tr>
<tr>
<td>Other medications</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antifungal (nilstat, nystatin, fluconazole)</td>
<td>56.3‡</td>
<td>46.2‡</td>
<td>14.3‡</td>
<td>0.0‡</td>
</tr>
<tr>
<td>Methylxanthine (aminophylline, theophylline)</td>
<td>37.5‡</td>
<td>29.3‡</td>
<td>17.9</td>
<td>0.0‡</td>
</tr>
<tr>
<td>Inotrope (dopamine, dobutamine)</td>
<td>28.1*</td>
<td>0.0†</td>
<td>3.3†</td>
<td>0.0†</td>
</tr>
<tr>
<td>Steroid (hydrocortisone)</td>
<td>3.1</td>
<td>0.0†</td>
<td>0.0†</td>
<td>0.0†</td>
</tr>
<tr>
<td>Diuretic (furosemide)</td>
<td>34.4*</td>
<td>7.7†</td>
<td>3.3†</td>
<td>0.0†</td>
</tr>
<tr>
<td>NSAID (indomethacin)</td>
<td>34.4*</td>
<td>2.3†</td>
<td>0.0†</td>
<td>0.0†</td>
</tr>
</tbody>
</table>

Significant differences (P < 0.05) between groups are indicated by symbols: *vs. all other groups; †vs. group D; ‡vs. group C; §vs. group B; ¶vs. group A.
Fig. 1. Urine output (A–C), creatinine clearance (D–F), and the fractional excretion of sodium (G–I) in neonates born at \( \geq 28 \) wk of gestation (group A), 29–31 wk of gestation (group B), 32–36 wk of gestation (group C), and \( \geq 37 \) wk of gestation (group D) on postnatal days 3–7 (top row; group A, \( n = 33 \); group B, \( n = 44 \); group C, \( n = 29 \); days 14–28 (middle row; group A, \( n = 23 \); group B, \( n = 19 \); group C, \( n = 7 \); and (bottom row) day 3 (group A, \( n = 34 \); group B, \( n = 44 \); group C, \( n = 30 \); group D, \( n = 20 \)) vs. day 28 (group A, \( n = 32 \); group B, \( n = 24 \); group C, \( n = 9 \); group D, \( n = 11 \)). Data were analyzed using a 2-way ANOVA (with repeated measures for line graphs) with the factors gestational age (pGA), postnatal age (pPA), and their interaction (pGAxPA). From Bonferroni post hoc analysis: \(^* P < 0.01 \) compared with groups A, B, and C (C and F). \( P < 0.05 \), a vs. b and c, b vs. c (F and I).

Inversely associated with gestational age at birth (Fig. 2). There was no change in urine protein levels with increasing postnatal age. There was wide intragroup variability in urine protein levels; within group A for example, UTP:Cr levels ranged from 92.2 to 759.3 mg/mmol at postnatal day 28 (Fig. 2A). In term infants, UTP:Cr (mean: 219.3 ± 139.9 mg/mmol) and albumin:Cr (mean: 19.9 ± 9.1 mg/mmol) levels measured on postnatal day 3 were similar to the levels found among group B neonates at postnatal day 7; \( \beta \)-M:Cr levels (mean: 0.8 ± 0.2 mg/mmol), however, were negligible. Pathological proteinuria (UTP \( \geq 500 \) mg/l) was observed in 12 (9.3%) neonates, at one or more postnatal time points, with the majority of these in group A (7/12), followed by group B (2/12), group C (1/12), and group D (2/12).

Urinary NGAL. There was a significant effect of gestational age on urinary NGAL, with the lowest NGAL levels observed at postnatal day 28 in group D term neonates (Fig. 3A). Nine preterm neonates (7.0%) had levels of urinary NGAL \( > 390 \) ng/ml (\( > 2 \) SD from the mean) at one or more postnatal time points, with the majority (7/9) occurring on postnatal day 28.

There was a significant positive linear correlation between urinary NGAL levels and UTP:Cr (Fig. 3B); however, urinary NGAL levels were not correlated with serum creatinine, CCr, FENa, urine albumin, or \( \beta \)-M levels (data not shown). Urinary NGAL levels in the subset of preterm neonates who exhibited pathological proteinuria (UTP \( \geq 500 \) mg/l) were not different from the NGAL levels of age- and sex-matched controls with low protein excretion at corresponding postnatal time points (Fig. 3C). In these two groups, with the exception of a few neonates, urinary NGAL levels were not predictive of proteinuria (Fig. 3D); urinary NGAL levels were not significantly increased at the time points before or at the onset of pathological proteinuria.

Impaired renal function. Overall, \( \sim 14\% \) of preterm neonates from each gestational age group were classified as having low

Fig. 2. Urine total protein (UTP; A), albumin (B), and \( \beta \)-2-microglobulin (\( \beta \)-M; C) levels corrected for urine creatinine, on postnatal days 7, 14, 21, and 28 in preterm neonates (groups A, \( n = 33 \); B, \( n = 40 \); and C, \( n = 25 \)). Data were analyzed using a 2-way ANOVA with the factors pGA, pPA, and pGAxPA.
urine output (Fig. 4A). Twenty-five percent of neonates in group A, 9.5% in group B, and 7.1% in group C had high serum creatinine levels (>100.6 μmol/l); only one neonate (group B), however, was categorized as having low C_{Cr} (Fig. 4, B and C). High FENa was predominantly observed in group A neonates, with three preterm neonates from groups A and B exhibiting hyponatremia (serum sodium levels <130 mmol/l) during the study period (Fig. 4D). High UTP levels were only observed in group B and group C neonates, whereas high urinary NGAL levels were observed in a small percentage of neonates in each gestational age group (Fig. 4, E and F).

The relationship between the six different measures of renal dysfunction (described in Fig. 4) and whether any preterm neonates had multiple measures of renal impairment were also examined. Twelve (11.2%) preterm neonates had more than two measures of renal impairment. As shown in Table 3, the most common combination of renal dysfunction measures exhibited during the first month of life was high serum creatinine and high FE_{Na} (observed in 7 of 12 neonates).

**DISCUSSION**

The findings of this study demonstrate that renal function in preterm neonates, during the first month of life, is significantly affected by gestational age at birth and postnatal age. Together, these results suggest that neonatal renal function is predominantly influenced by renal structural maturity. By postnatal day 28, C_{Cr} was significantly lower among preterm neonates compared with term infants; however, differences in FE_{Na} were not observed, which is suggestive of a high capacity for postnatal tubular maturation. Both urinary protein and NGAL levels were inversely associated with gestational age at birth, which suggests that they are markers of renal immaturity. Of concern, pathological proteinuria was observed in 12 preterm neonates; among those neonates, urinary NGAL levels were not elevated, which has two potential implications: 1) that the cause of the pathological proteinuria is not due to acute kidney injury (as NGAL has previously been shown to be a marker of AKI); or 2) if the pathological proteinuria observed is due to renal injury, urinary NGAL is not a useful marker or predictor of renal injury in this population. The findings from this study provide normative values for a number of renal functional parameters in preterm infants. The presence of renal dysfunction in a high proportion of babies highlights the importance of conducting large-scale studies among populations of preterm neonates and the close monitoring of renal function in preterm infants in the neonatal intensive care unit (NICU).

**Maturation of Renal Function in the Early Postnatal Period**

In this study, urine output was not significantly different during the first week of life among preterm neonates. This finding was expected, as within the NICU fluid intake is strictly maintained according to neonatal body weight, and from approximately postnatal day 4 urine output is known to be predominantly influenced by fluid intake (26, 27). From postnatal day 14 to postnatal day 28, a significantly higher urine output was observed among neonates born at older gestational ages, which likely relates to the change in feeding regime. All term infants were exclusively breast-fed in the first week after...
birth; hence their observed low urine output is likely due to low maternal milk production within the first few days after birth. Since this was a noninvasive study, urine output was estimated using diaper weights; it is important to note that this method has its limitations since estimates based on averages had to be used in occasional instances of missed voids and soiled diapers. The 24-h urine collections conducted at home were also highly reliant on the record-keeping of parents, and this noninvasive technique was therefore prone to error. Furthermore, the number of infants assessed after postnatal day 7 was reduced due to loss to follow-up; therefore, the robustness of data was diminished for all analyses conducted on postnatal days 14–28. Consistent with previous studies, as gestational age at birth and postnatal age increased, CCr increased (2, 5, 7–9, 12, 14, 21, 39, 47, 51, 56) and FENa decreased (2, 12, 13, 39, 47). Although neonates born very or extremely preterm commence with a low GFR and high FENa, by postnatal day 28 both CCr and FENa were not significantly different between the preterm gestational age groups, which is suggestive of a high capacity for postnatal glomerular and tubular maturation among those born very or extremely preterm. Interestingly, the rate of change in CCr over the first month of life was very similar between all groups of neonates, and in contrast to previous studies (5, 14), a slower postnatal increase in CCr in preterm neonates compared with term infants was not observed. In extremely preterm neonates, there was a significantly greater rate of change in FENa from postnatal day 3 to day 28 compared with all other groups, suggestive of more accelerated tubular maturation. In support of this idea, at postnatal day 28 CCr remained significantly lower among

Table 3. Measures of renal dysfunction in 12 preterm neonates that exhibited multiple (≥2) measures of renal dysfunction, by gestational age grouping and sex

<table>
<thead>
<tr>
<th>Gestational Age Group</th>
<th>Sex</th>
<th>Low Urine Output</th>
<th>Low CCr</th>
<th>High Serum Cr</th>
<th>High FENa</th>
<th>High UTP</th>
<th>High NGAL</th>
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<td>A (≤28 wk)</td>
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<td>C (32–36 wk)</td>
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<td>C (32–36 wk)</td>
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<td>C (32–36 wk)</td>
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CCr, creatinine clearance; FENa, fractional excretion of sodium; NGAL, neutrophil gelatinase-associated lipocalin.
Evidence of Renal Dysfunction in Preterm Neonates

In this study, renal impairment was defined as measures of renal function that were >2 SD from the mean. There is a current lack of definition in the literature as to what constitutes AKI in the preterm neonate; if the general definition of AKI commonly used in adults [RIFLE criteria (4, 30)] was applied in the current study, only one preterm neonate (and 10 term neonates presenting with oliguria) clearly met the criteria. Therefore, we adopted a broader definition of dysfunction in the current study to give an indication as to the percentage of infants with reduced renal functional capacity (glomerular and tubular), rather than focusing on the strict AKI criteria.

In the present study, 25% of neonates in group A, and <10% in groups B and C, exhibited high serum creatinine levels (>2 SD from the mean); there was just one preterm neonate (group B), however, that had low C\textsubscript{Cr} levels (day 3–6 of life). A relatively high percentage of preterm neonates, predominantly those born extremely preterm, were observed to have a high percentage of sodium excretion; however, likely due to the administration of sodium supplementation in this population, the majority of preterm neonates maintained adequate levels of serum sodium. It may be speculated that the high sodium excretion in these neonates represents either tubular immaturity or injury. Of the neonates that exhibited more than one measure of renal dysfunction, the majority had both high \( F\text{ENa} \) and high serum creatinine. If tubular function is impaired, this may also have an impact on serum creatinine levels as creatinine (besides predominantly being filtered by the glomeruli) is known to be actively excreted by proximal tubular cells (25, 35), and in the case of preterm neonates, may also be reabsorbed by the immature tubules as has been observed in a neonatal animal model (29). Serum creatinine levels are also influenced by extrarenal factors such as muscle mass, and the intake of nitrogen, protein, and creatinine (25, 35) which is increased with milk formula and other parenteral nutrition preparations (37). Each of these factors may have influenced the relatively high proportion of neonates who also exhibited high serum creatinine levels; in contrast, only one preterm neonate was found to have low C\textsubscript{Cr}. In general, C\textsubscript{Cr} is considered to be a more reliable indicator of GFR than serum creatinine levels alone (46), especially given the large number of factors that may influence the generation of creatinine as described above. Encouragingly, this finding may indicate that the capacity for glomerular filtration in the preterm kidney in the early neonatal period is quite adequate; however, tubular function is likely impaired.

Pathological proteinuria was observed among 12 neonates in this study (including 2 term-born infants) at 1 or more postnatal time points. In this regard, the only preterm neonate with low C\textsubscript{Cr} also exhibited pathological proteinuria on postnatal days 14, 21, and 28. Interestingly, the onset of severe proteinuria was often observed at a later stage (9/12 neonates exhibited pathological proteinuria after day 21), which suggests that factors in the postnatal clinical course (rather than renal immaturity) may be the cause of the severe proteinuria. In support of this idea, it was only neonates born at older gestational ages that exhibited high UTP levels (>2 SD from the mean). Exposure to nephrotoxic medications (including NSAIDs and antibiotics) are known to impair nephrogenesis (42, 49) and cause renal injury, such as podocyte foot process effacement (22). It is important to note that with regard to all the maternal and fetal factors we examined (including medications), there was no direct correlation with renal impairment, thus indicating that the renal dysfunction is likely to be multifactorial in origin. Future studies in a larger cohort of neonates are required to identify individual factors (such as exposure to medications and growth restriction) which may be contributing to renal dysfunction following preterm birth.

Urinary NGAL as a Marker of Acute Kidney Injury

NGAL is excreted by renal proximal tubule cells as a response to AKI (41); however, NGAL is produced during nephrogenesis (16), and levels may also be raised in late-onset sepsis (34). Although positive findings have been reported among older infants (57), the usefulness of urinary NGAL as a marker of AKI in preterm neonates remains unclear (20, 23, 33, 34). Certainly, consistent with previous studies (20, 23) we found a significant inverse correlation between urinary NGAL and gestational age at birth; these findings may relate to the immaturity of the kidney, and the clinical instability of the younger neonates. Importantly, the results of the current study demonstrated that urinary NGAL levels were not directly correlated with any indicators of renal dysfunction, apart from UTP. Although the correlation between NGAL and UTP was statistically significant, it is to be noted that the coefficient of determination was very low. Furthermore, only two preterm neonates were observed to have concurrently high UTP and NGAL levels; in general, neonates with pathological proteinuria did not exhibit high urinary NGAL levels. There was also no association between NGAL levels at time points before or at the time of...
proteinuria onset, suggesting that NGAL may not be predictive of renal injury and/or proteinuria.

Conclusions

The findings of this study demonstrate that renal maturity is an important determinant of glomerular and tubular function among preterm neonates. Of particular concern, a number of preterm neonates exhibited severe proteinuria; however, there was no correlation with urinary NGAL levels. Given the immaturity of the preterm kidney, it is important to determine what levels of protein in the urine are normal vs. those that are pathological and thus indicative of renal injury in preterm neonates. Furthermore, it would be of value to identify specific factors in the postnatal clinical care of the preterm neonate which may be leading to the high urinary protein excretion. The results from this study have been important in working toward the development of a normal range of urinary protein levels in preterm neonates, but our findings are limited by the relatively small sample size and the large inter- and intragroup variability observed. The consequences of proteinuria in the neonatal period are unknown; however, the potential for progressive renal injury and long-term renal dysfunction suggests the need for regular assessments of renal function in subjects that are born preterm.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

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