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ORIGINAL ARTICLE

Urinary protein to creatinine ratio during the first month of life in very preterm infants—a prospective cohort study (PROTIPREMA)

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Abstract

Background Preterm infants have physiological proteinuria and values of urine protein to creatinine ratio (UPr/Cr) are higher compared to full-term infants during the first week of life. Few investigations explored the changes of proteinuria in very preterm infants (VPI, ≤ 31 weeks of gestation) older than a week, and it is unclear whether high and persistent proteinuria is associated with kidney injury in this population. This study aimed to (1) observe the changes of UPr/Cr during the first month of life in VPI and (2) describe clinical and biological variables associated with the changes of UPr/Cr.

Methods Spot urine samples for UPr/Cr were collected on the first day of life (DOL1) and then on DOL2-3, DOL5-6, second week of life (WOL2), WOL3, and WOL4 in VPI cared for in a third-level NICU. We tested the relationship of UPr/Cr with perinatal variables and diseases.

Results A total of 1140 urine samples were obtained for 190 infants. UPr/Cr values (mg/mmol) (median with interquartile) at DOL1, DOL2, DOL3, WOL2, WOL3, and WOL4 were, respectively, 191 (114–399), 226 (152–319), 225 (156–350), 282 (200–488), 308 (188–576), and 325 (175–664). At the multivariate analysis, lower gestational age (GA) and increasing postnatal age were the only variables significantly associated with higher UPr/Cr values ($p < 0.001$). There was wide intra- and interindividual variability in UPr/Cr, especially in infants with higher GA and clinical stability.

Conclusions In VPI, UPr/Cr is higher at lower GA and increases with advancing postnatal age. High persistent proteinuria is not associated with clinical and biological variables reflecting kidney injury during the first month of life.

Keywords Extremely low birth weight · IUGR · AKI · Creatinine · Hyperfiltration · Proteinuria · Congenital nephron deficit

Introduction

Proteinuria is a common finding on urinalysis in neonates and young infants. In the majority of cases, the presence of proteins in urine is benign, transient, and physiological. However, elevated urinary concentrations of proteins can be a marker of underlying kidney injury, and persistent proteinuria must be checked when assessing signs of kidney damage. In both clinical practice and for research purposes, spot, random urine tests for protein to creatinine ratio (UPr/Cr) are validated methods for measuring proteinuria, as they are well-correlated to 24-h urine protein output. Normal values of UPr/Cr have been established at < 20 mg/mmol in adults and children over 2 years of age [1] and at < 50 mg/mmol in younger infants aged 6 to 24 months [2]. Protein to creatinine ratio is higher in full-term neonates, for whom upper limit values of UPr/Cr have been established at 136 mg/mmol on the third day of life [3]. Even higher levels of UPr/Cr have been reported in preterm infants during the first days of life, as one recent study determined the upper limit values at 223 and 289 mg/mmol, respectively, on days 0–1 and 3–4 of life, in a large cohort of newborn infants born before 37 weeks of gestation [4].

Compared with babies born at term, preterm neonates have slower progression in kidney maturation after birth, for both glomerular and tubular function [5, 6]. The increased urinary protein levels reported in preterm neonates have been

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claimed to be caused by a more severe kidney immaturity and numerous studies agree that proteinuria (total amount) and UPr/Cr are inversely correlated with gestational age at birth, and during the first days of life [4, 7, 8]. Very preterm infants (VPI, born at less than 32 weeks of gestation) have a reduced nephron number and are often exposed to several antenatal and postnatal factors (including acute kidney injury (AKI)) that contribute to worsen the nephron deficit [9]. Due to glomerular and tubular immaturity, proteinuria is expected in these patients during the early postnatal period, but it would be of interest to explore whether, in this context, high and persisting proteinuria can be an indicator of kidney injury and can be associated with risk factors of impaired kidney function. To our knowledge, few investigations have explored the evolution of protein excretion in preterm infants after the first week of life, with conflicting results [8, 10, 11], and no study has extensively described the maturational changes of UPr/Cr during the first month of life in neonates born very preterm.

The present investigation was carried out with the aim to describe the changes of UPr/Cr during the first month of life in preterm neonates with gestational age ≤ 31 weeks of gestation. Our secondary aim was to identify clinical and biological factors associated with the changes of UPr/Cr in this cohort.

Methods

This was a prospective observational study conducted in a third-level NICU of the Reunion Island University Hospital (France) from March 2018 to February 2020.

Participants and study protocol

All infants born at less than 32 weeks of gestation without antenatal diagnosis of kidney defects and/or malformations were eligible. Infants were recruited during the first 24 h after birth. For each patient, the first urine sample was collected on the first day of life (DOL1) and then as follows: DOL2-3, DOL5-6, second week of life (WOL2), WOL3, and WOL4. Two sterile gauze pads were placed in the infant nappies, removed no more than 3 h later, and then put into a syringe without needle. The urine was collected by pushing the syringe plugging in a dry tube. Collected urines were immediately stored at − 80 °C until analysis. Samples soiled by meconium were discarded. The urine total protein was measured by the acid turbidimetry method, and the urinary creatinine was measured by multi-enzymatic assay (COBAS ® 6000 automatic analyzer (Roche, Germany)). UPr/Cr was expressed as mg/mmol. Daily urine output was assessed every 6 h on the day of the sample collection, by weighing nappies, and expressed as ml/kg/h.

Data collection

Information was collected about antenatal history, pregnancy morbidities, and mode of delivery. The following data about birth and postnatal diseases were also collected: gestational age, birth weight, gender, appropriateness of birth for gestational age (according to reference [12]), 1- and 5-min Apgar, severe birth asphyxia (umbilical cord pH ≤ 7 and/or lactates ≥ 11 mmol/l), acute anemia at birth (venous hemoglobin < 13.3 g dl^{-1} at admission into NICU), early-onset sepsis (EOS), respiratory distress syndrome (RDS) requiring surfactant, AKI (absolute serum creatinine > 130 µmol/l after the first day of life) [13], hemodynamically significant patent ductus arteriosus ((HsPDA), PDA needing clinical or surgical treatment), hypotension requiring treatment, late-onset sepsis, bronchopulmonary dysplasia (BPD), intraventricular hemorrhage (IVH) graded at cerebral ultrasound according to Papile et al. [14], cystic periventricular leukomalacia (PVL) at cranial ultrasound [15], and death before discharge from hospital. We collected data on urine output, serum creatinine, protein, and electrolyte levels during the study period. Dietary protein intakes were recorded. Data on drugs administered during the hospital stay were also collected.

Outcome measures and statistical analysis

Categorical variables are presented as frequencies, and continuous variables as means ± standard deviations. The normality of distribution was checked using the Shapiro–Wilk test. Data of UPr/Cr were non-normally distributed and are presented as median with interquartile range (25–75%).

The main outcome of interest was the maturation of the UPr/Cr during the first month of life. With the purpose of the secondary outcome, we tested the relationship of UPr/Cr with perinatal variables, drugs, AKI as defined above, and postnatal diseases. First, bivariate comparisons were performed using the $\chi 2$ test or Fisher exact test for qualitative variables, and ANOVA or the Kruskal–Wallis test, if appropriate, for continuous variables. Second, to estimate the independent association with UPr/Cr, we performed a linear multiple regression. All factors associated with UPr/Cr with a p value < 0.1 in bivariate analyses were included in the multivariate linear regression. A backward step-wise selection was then applied to obtain the final model.

The variability of the UPr/Cr in the population (interindividual) and in the same patient (intraindividual) were assessed by the coefficient of variation (CV) [16]. Study patients were categorized into 2 groups according to the

intraindividual CV: low variability (CV ≤ 100%) and high variability (CV > 100%). We performed a multivariate logistic regression in order to analyze factors associated with the condition of having a low variability.

A p value < 0.05 was considered significant. All analyses were performed using MedCalc software (version 12.3.0; MedCalc Software, Ostend, Belgium).

Ethics statement

This study was registered on ClinicalTrials.gov under the number NCT04608279. The research protocol was approved by the National Ethics Committee (CPP EST I, 2017-A02700-53). Informed parental consent was obtained for all the patients included in the study before any urine collection.

Results

Overall, 190 infants were included, and a total of 1140 urine samples were available from days 1 to 28 of life. Table 1 shows the baseline characteristics and morbidities of the study population. Data on postnatal drug exposure in the study population are presented in Table 2.

Table 1 Baseline characteristics and morbidities of 190 preterm infants born at less than 32 weeks of gestation

Characteristics	
Gestational age, weeks, mean (±SD)	29.0 (2.1)
Birth weight, g, mean (±SD)	1132 (336)
Male gender (%)	102 (53.7)
Small for gestational age (%)	24 (12.6)
Inborn (%)	181 (95%)
Cesarean section (%)	133 (70)
1-min Apgar, seconds (±SD)	6.5 (3.2)
5-min Apgar, seconds (±SD)	8.0 (2.4)
Severe birth asphyxia (%)	8 (4.2)
Acute anemia at birth (%)	15.4 (1.9)
RDS requiring surfactant (%)	110 (58)
Treated hypotension (%)	67 (35)
Early-onset sepsis (%)	16 (8.4)
Late-onset sepsis (%)	31 (16.3)
HsPDA (%)	51 (27)
AKI (%)	10 (5.3)
IVH (stage 1–2) (%)	35 (18.4)
IVH (stage 3–4) (%)	14 (7.4)
PVL (%)	5 (2.6)
BPD at 36 weeks of GA (%)	21 (11)
Death (%)	16 (8.4)

Values are expressed as number (%), unless indicated otherwise

RDS, respiratory distress syndrome; *AKI*, acute kidney injury; *HsPDA*, hemodynamically significant patent ductus arteriosus; *BPD*, bronchopulmonary dysplasia; *IVH*, intraventricular hemorrhage; *PVL*, periventricular leukomalacia

Table 2 Postnatal drug exposure in the study population (190 preterm infants born at less than 32 weeks of gestation)

Drug	*N* (%)
Nephrotoxic antibiotics at birth (gentamicin)*	125 (65.8)
Nephrotoxic antibiotics during hospital stay (gentamicin or vancomycin)**	19 (10)
Vasopressors (dopamine, dobutamine, or nor-adrenaline)	41 (21.6)
Non-steroidal anti-inflammatory drugs (ibuprofen)	48 (25.3)
Early hydrocortisone for preventing DBP	33 (17.4)
Hydrocortisone for treating hypotension	4 (2.1)

Values are expressed as number (%)

*Prevention or management of early-onset neonatal sepsis. **Suspected or confirmed late-onset sepsis

Values of UPr/Cr from birth to the first month of life are presented in Table 3 and Fig. 1. As expected, the distribution of values was not normal ($p < 0.0001$).

At the univariate analysis, the following variables were significantly associated with UPr/Cr: gestational age, postnatal day, male gender, acute anemia at birth, RDS requiring surfactant, AKI, HsPDA, BPD, IVH, and death before discharge from hospital (data not shown). They were entered in the multivariate regression model. The multivariate analysis showed that UPr/Cr was significantly higher at lower gestational ages and increased significantly with advancing postnatal day. There was a borderline significance for HsPDA, being associated with lower values of UPr/Cr (Table 4). UPr/Cr according to gestational age and changes of the UPr/Cr in relation to birth at less or more than 28 weeks of gestation are shown in Fig. 2.

Intraindividual variability was high in 46 (24.5%) and low in 144 (75.5%) infants. Median values (25–75°) of UPr/Cr were 262 (167–417) and 233 (143–641) mg/mmol in the low and in the high variability group, respectively ($p = 0.93$). UPr/Cr changes in infants with low and high intraindividual CV are illustrated in Figs. 3 and 4. The following variables were associated with the condition of having a low variability: low GA at birth, HsPDA, IVH, AKI, EOS, treated hypotension, and BPD free-survival. Among these factors, HsPDA, BPD free-survival, and IVH remained significantly associated with the condition of low variability upon multivariate analysis (Table 5).

Discussion

This is the first prospective report illustrating the changes of urine total protein to creatinine ratio during the first month of life in a large cohort of very preterm babies. Our study demonstrates that, starting on the first day of life, an increase

Table 3 Values of UPr/Cr from birth to the fourth week of life in 190 preterm infants born at less than 32 weeks of gestation

	DOL1	DOL2-3	DOL4-5	WOL2	WOL3	WOL4
UPr/Cr (mg/mmol)	191 (114–399)	226 (152–319)	225 (156–350)	282 (200–488)	308 (188–576)	325 (175–664)

Values are presented as median with interquartile range (25–75%)

UPr/Cr, urinary protein to creatinine ratio; *DOL*, day of life; *WOL*, week of life

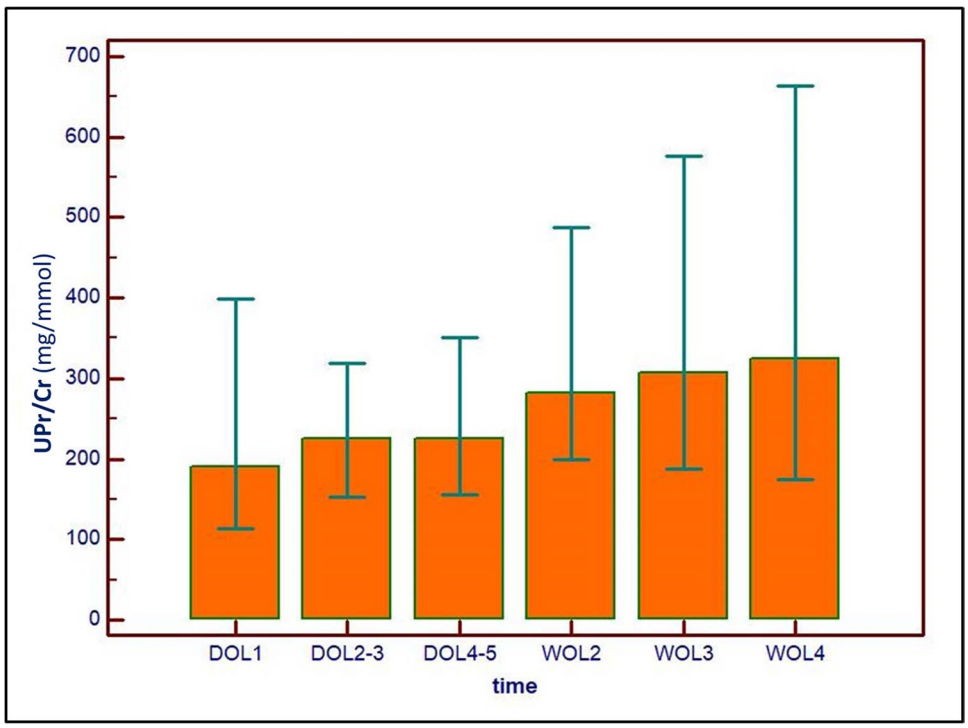

Fig. 1 Distribution of UPr/Cr (mg/mmol) from birth to the fourth week of life. Values are presented as median with interquartile range (25–75%), $p < 0.001$

Table 4 Results of multivariate analysis for UPr/Cr (linear multiple regression, $R.^2 = 0.04218$, $p < 0.001$)

Variables	Coefficient	Std. error	$r_{partial}$	p
(Constant)	1599.1901			
GA	−45.4472	13.1839	−0.1067	0.0006
Postnatal day	17.2502	2.8123	0.1876	< 0.0001
HsPDA	−119.1318	62.4004	−0.05935	0.0565

GA, gestational age; *HsPDA*, hemodynamically significant patent ductus arteriosus

of UPr/Cr is detectable in immature kidneys under different pathophysiological conditions and that this increase persists throughout the first month of life. In accordance with previous studies, in which, however, the urine sample was performed during the first days of life and data collection was not extended over 4 weeks [4, 7, 17], we confirmed that, starting at birth, the urinary proteinuria (UPr/Cr) is inversely correlated with gestational age. In contrast with other works [4, 7, 17, 18], we did not find any association between urine protein excretion and maternal and infant morbidities, AKI, illness severity, or postnatal drug exposure. In our cohort, UPr/CR significantly increased with advancing postnatal age. Such findings are in contrast with those of Ojala et al. [11]. In a cohort of 100 infants of 24–32 weeks of gestation, they reported that the highest peaks of tubular proteinuria occurred at a median of 5 days of life and proteinuria normalized thereafter, except for infants with prolonged vancomycin treatment and lower gestational age.

A possible explanation of these contrasting results could be that different urinary proteins have been analyzed across these reports, namely proteins with molecular weight higher (HMW) and lower (LMW) than albumin, or tubular proteinuria (urinary alpha-1-microglobulin) [11].

Urinary protein excretion depends on the passage of protein (including macromolecules and albumin) across the glomerular filtration barrier into the filtrate and by the uptake of filtered protein in the proximal tubule. Traditionally, the proteinuria of the healthy neonate has been explained by the "physiological" leak of low molecular weight protein by the immature proximal tubule. In preterm critically ill infants,

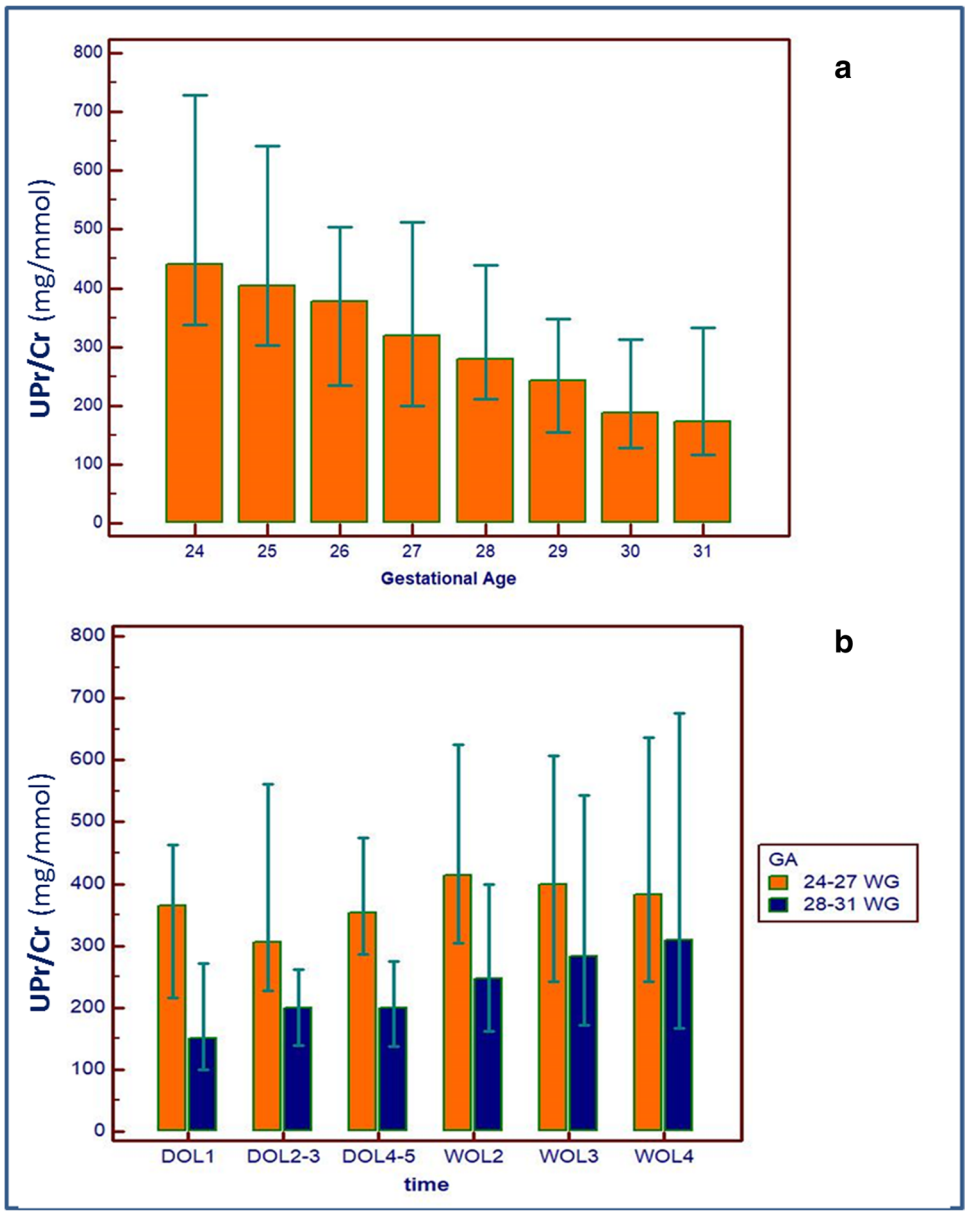

Fig. 2 **a** Values of UPr/Cr according to GA ($p < 0.01$). **b** Maturation of the UPr/Cr in relation to birth at less or more than 28 weeks of gestation ($p < 0.01$). Values are presented as median with interquartile range (25–75%)

both glomerular filtration and tubular reabsorption can be impaired in cases of delayed maturation or acute kidney injury. In our study, fractional differentiation and quantitative measurement of urinary protein were not performed; therefore, our findings do not allow us to make conclusions about the glomerular or the tubular origin of the excreted protein.

In our cohort, very high levels of UPr/Cr and persistent proteinuria, especially in some extremely low birth weight infants, were not correlated with impaired kidney function (as measured by the occurrence of AKI) or with risk factors for AKI. This is consistent with the findings of Gubhaju and colleagues [8]. In their study, like in ours, there was a very high inter- and intraindividual variability of urinary protein excretion. Interestingly, in our cohort, a higher intraindividual variability was observed in more mature infants, with higher gestational age and with clinical stability, whereas infants of lower gestational age and with critical illness showed a low variability. We speculate that the degree of variability could change throughout the maturation process of kidney protein handling. However, we did not look at markers of glomerular and tubular maturation in our cohort and we did not collect urine samples at term of corrected age, and this limits the interpretation of our results. Moreover, even if we did not find any association between protein intakes and urinary protein excretion in this study, we cannot exclude that other specific factors of the clinical care that we did not consider (i.e., water intake and nutrient intake other than proteins) may influence protein excretion in preterm infants cared for in the NICU.

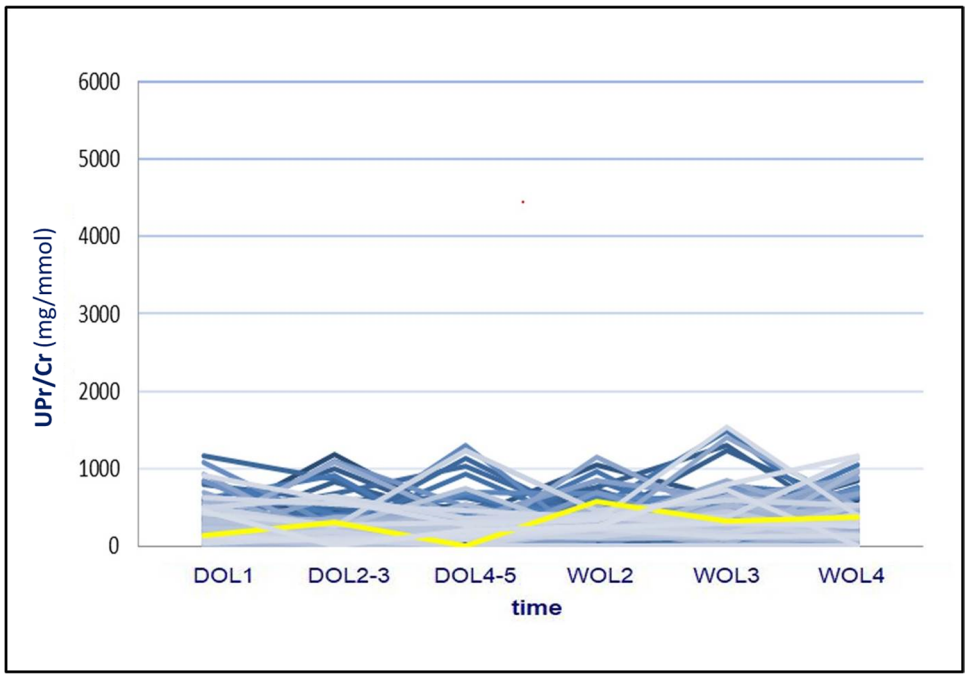

Fig. 3 Changes of UPr/Cr from birth to the fourth week of life in 144 infants (75.5% of the study cohort), with low intraindividual coefficient of variation (CV ≤ 100%)

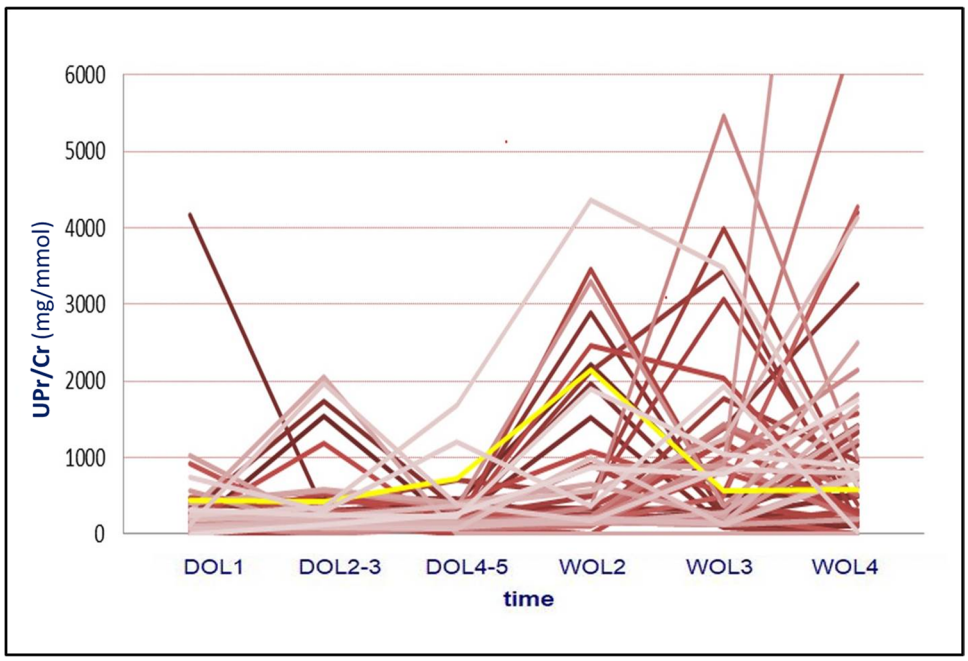

Fig. 4 Changes of UPr/Cr from birth to the fourth week of life in 46 infants (24.5% of the study cohort), with high intraindividual coefficient of variation (CV > 100%)

By comparison with previous studies in preterm and at term newborn infants [7, 10], we did not perform a continuous urine collection, which can be cumbersome and complicated in pediatrics. As in Ponthier et al.'s study [4], we measured protein excretion on spot urine sample and we normalized for urine creatinine. This can be subject to criticism, as striking variations in urinary creatinine excretion may occur in newborns, especially those born preterm [19] and in spot urine samples. Previous studies [7, 10] utilized adherent plastic bags for urine collection. These latter may pose practical problems, especially in VPI, as they may traumatize the skin, and are prone to leakage. We chose a different non-invasive technique, and we cannot exclude that this difference may have influenced the results. Nevertheless, the collection technique that we used in the present study has been validated in previous reports [3, 4] and urine protein to creatinine ratio remains a suitable method to study the urine protein excretion in large cohorts of neonates [8, 11]. Finally, considering the generalization of total protein to creatinine ratios

Table 5 Variables significantly associated with the condition of low variability of the UPr/Cr (logistic multiple regression, chi-square = 53.5683, $p < 0.0001$)

Variables	Coefficient	Std. error	*p*
(Constant)	−0.6769		
HsPDA	+0.70307	0.22537	0.0018
BPD free-survival	−1.25438	0.32467	0.0001
IVH	+0.94860	0.21536	<0.0001

HsPDA, hemodynamically significant patent ductus arteriosus; *IVH*, intraventricular hemorrhage; *BPD*, bronchopulmonary dysplasia

in clinical practice, results of our study will be useful in working toward a definition of normal ranges of these values in preterm infants during the first month of life.

Conclusion

In conclusion, we have reported the changes of UPr/Cr in a large cohort of preterm infants born at less than 32 weeks of gestation and cared for in a NICU. Our results suggest that high persistent proteinuria may be indicative of kidney immaturity in very low birth weight infants and also that, during the first month of life, proteinuria is not associated with clinical and biological variables reflecting kidney injury in these infants.

Both the maturation of proteinuria after the first month of life and the long-term consequences on kidney function of high persistent proteinuria deserve further investigations in very preterm infants.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00467-022-05653-8.

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Declarations

Conflict of interest The authors declare no competing interests.

References

1. Hogg RJ, Portman RJ, Milliner D, Lemley KV, Eddy A, Ingelfinger J (2000) Evaluation and management of proteinuria and nephrotic syndrome in children: recommendations from a pediatric nephrology panel established at the National Kidney Foundation conference on proteinuria, albuminuria, risk, assessment, detection, and elimination (PARADE). Pediatrics 105:1242–1249. https://doi.org/10.1542/peds.105.6.1242
2. Hogg RJ, Furth S, Lemley KV, Portman R, Schwartz GJ, Coresh J, Balk E, Lau J, Levin A, Kausz AT, Eknoyan G, Levey AS (2003) National Kidney Foundation's Kidney Disease Outcomes Quality Initiative clinical practice guidelines for chronic kidney disease in children and adolescents: evaluation, classification, and stratification. Pediatrics 111:1416–1421. https://doi.org/10.1542/peds.111.6.1416
3. El Hamel C, Chianea T, Thon S, Lepichoux A, Yardin C, Guigonis V (2017) Normal values of urine total protein- and albumin-to creatinine ratios in term newborns. Pediatr Nephrol 32:113–118. https://doi.org/10.1007/s00467-016-3427-0
4. Ponthier L, Trigolet M, Chianea T, Mons F, Yardin C, Guigonis V, El Hamel C (2021) Distribution of proteinuria- and albuminuria-to-creatinine ratios in preterm newborns. Pediatr Nephrol 36:1515–1524. https://doi.org/10.1007/s00467-020-04838-3
5. Iacobelli S, Guignard JP (2021) Maturation of glomerular filtration rate in neonates and infants: an overview. Pediatr Nephrol 36:1439–1446. https://doi.org/10.1007/s00467-020-04632-1
6. Gattineni J, Baum M (2015) Developmental changes in renal tubular transport—an overview. Pediatr Nephrol 30:2085–2098. https://doi.org/10.1007/s00467-013-2666-6
7. Clark PM, Bryant TN, Hall MA, Lowes JA, Rowe DJ (1989) Neonatal renal function assessment. Arch Dis Child 64:1264–1269. https://doi.org/10.1136/adc.64.9.1264
8. Gubhaju L, Sutherland MR, Horne RS, Medhurst A, Kent AL, Ramsden A, Moore L, Singh G, Hoy WE, Black MJ (2014) Assessment of renal functional maturation and injury in preterm neonates during the first month of life. Am J Physiol Renal Physiol 307:F149–F158. https://doi.org/10.1152/ajprenal.00439.2013
9. Iacobelli S, Guignard JP (2022) When the progresses in neonatology lead to severe congenital nephron deficit: is there a pilot in the NICU? Pediatr Nephrol 37:1277–1284. https://doi.org/10.1007/s00467-021-05338-8
10. Galaske RG (1986) Renal functional maturation: renal handling of proteins by mature and immature newborns. Eur J Pediatr 145:368–371. https://doi.org/10.1007/BF00439240
11. Ojala R, Ala-Houhala M, Harmoinen AP, Luukkaala T, Uotila J, Tammela O (2006) Tubular proteinuria in pre-term and full-term infants. Pediatr Nephrol 21:68–73. https://doi.org/10.1007/s00467-005-2028-0
12. Growth Calculator for Preterm Infants. http://www.peditools.org/fenton2013. Accessed April 2022
13. Guignard JP, Ali US (2016) Acute renal failure in the neonate. J Pediatr Intensive Care 5:42–49. https://doi.org/10.1055/s-0035-1564735
14. Papile LA, Burstein J, Burstein R, Koffler H (1978) Incidence and evolution of subependymal and intraventricular hemorrhage: a study of infants with birth weights less than 1500 gm. J Pediatr 92:529–534. https://doi.org/10.1016/s0022-3476(78)80282-0
15. deVries LS, Eken P, Dubowitz LMS (1992) The spectrum of leukomalacia using cranial ultrasound. Behav Brain Res 49:1–6. https://doi.org/10.1016/s0166-4328(05)80189-5
16. Everitt BS, Skrondal A (2010) The Cambridge dictionary of statistics. Cambridge University Press, New York
17. Awad H, El-Safty I, El-Barbary M, Imam S (2002) Evaluation of renal glomerular and tubular functional and structural integrity in neonates. Am J Med Sci 324:261–266. https://doi.org/10.1097/00000441-200211000-00005
18. Sutherland MR, Chatfield MD, Davison B, Vojisavljevic D, Kent AL, Hoy WE, Singh GR, Black MJ (2019) Renal dysfunction is already evident within the first month of life in Australian Indigenous infants born preterm. Kidney Int 96:1205–1216. https://doi.org/10.1016/j.kint.2019.07.015
19. Matos V, Drukker A, Guignard JP (1999) Spot urine samples for evaluating solute excretion in the first week of life. Arch Dis Child Fetal Neonatal Ed 80:F240–F242. https://doi.org/10.1136/fn.80.3.f240