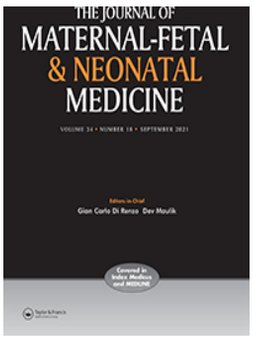

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ORIGINAL ARTICLE

Neutrophil gelatinase-associated lipocalin distribution in preterm newborns without acute kidney injury as defined by a reference method

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ABSTRACT

Introduction: Neutrophil gelatinase-associated lipocalin (NGAL) has been proposed as an early acute kidney injury (AKI) biomarker in the neonatal population. Our goal is to describe this biomarker behavior in this high-risk population, in absence of AKI as confirmed by inulin clearance.

Materials and methods: Prospective study including 42 preterm newborns (mean gestational age: 30.7 ± 2.3 weeks) with a urinary NGAL collection between day 1 and 6 of life.

Results: Median urinary neutrophil gelatinase-associated lipocalin (uNGAL) value is 122.8 ng/ml (7–1981.5 ng/ml). Statistically significant higher uNGAL values are found in female. uNGAL median values are decreasing when comparing extremely, very, and late preterm groups (812.2 ng/ml [75.8–1453.9] vs. 124.4 ng/ml [31.4–1981.5] vs. 65.3 ng/ml [7.1–1091]). There is a statistically significant inverse correlation between gestational age and uNGAL values (Pearson's coefficient $r = -0.37$). uNGAL median values are higher in groups exposed to gentamicin, neonatal asphyxia, early onset sepsis, or patent ductus arteriosus. Median inulin clearance is 18.8 ml/min/1.73 m^2 [14.8–25.5 ml/min/1.73 m^2]. There is no correlation between uNGAL values and inulin clearance results (Pearson's coefficient $r = -0.29$, p: .06).

Conclusions: In this preterm newborn's series without AKI, the median uNGAL and its high variability are in accordance with published reference ranges. Correlation between uNGAL and gestational age exists, as well as gender impact. Newborns exposed to different renal insults present higher uNGAL values, suggesting potential undetected tubular toxicity or reflecting NGAL production in case of inflammatory or ischemic processes.

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Infant; premature; acute kidney injury; neutrophil gelatinase-associated lipocalin; inulin clearance

Introduction

Neonatal acute kidney injury (AKI) affects 30–50% of very low birth weight infants. It is known as an independent risk factor for morbidity–mortality [1]. The neonatal KDIGO AKI definition in preterm newborn is based on increase of serum creatinine or decrease of urinary output [2]. This definition has several limitations. Indeed, serum creatinine reflects the mother's value in the first days of life and is also higher because of an increased tubular back diffusion. Furthermore, urinary output in preterm AKI is most often preserved due to the concentrating defect of immature tubules. Acute kidney injury in early life, associated with low nephron endowment secondary to preterm birth or intra-uterine growth restriction (IUGR) increases the risk of lifelong hypertension, cardiovascular disease, and chronic kidney disease. Identification of biomarkers improving the detection of acute renal injury is mandatory to improve the management of this special population and improve the adult prognosis [3].

Functional genomic studies identified neutrophil gelatinase-associated lipocalin (NGAL) as the earliest induced protein after renal ischemic injury in rat [4]. NGAL is a 26-kDa protein of the lipocalin family constitutionally expressed by several tissues and neutrophils. Its expression is upregulated during inflammation, infection, and ischemic events. NGAL is partially absorbed in the proximal tubule in normal conditions. In case of renal injury, NGAL is also produced by the loop of Henle and the collecting ducts, rising 6–12 h after the insult, proportionally to the severity of the aggression [5].

Pediatric studies have shown that urinary NGAL can predict, with a good performance, AKI and dialysis requirement in the post-operative pediatric cardiac surgery period [6–8]. Urinary neutrophil gelatinase-associated lipocalin (uNGAL), in the preterm population, has

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been proposed as an early AKI biomarker with moderate discriminative performance [9,10]. uNGAL reference range value in the preterm population has now started to be reported. However, uNGAL values in preterm population are influenced by other parameters as gestational age, gender, and neonatal complications in particular, sepsis and asphyxia [11–16].

Understanding this biomarker behavior and the associated variations, in this particular high-risk population, is crucial. Our goal is to describe uNGAL values in a series of premature newborns in the first days of life. In this study, no preterm presented AKI as confirmed by a reference method (inulin clearance).

Materials and methods

This report describes a prospective observational study performed in a neonatal intensive care unit in Geneva, Switzerland, from October 2012 to November 2015. The ethics review board approved this study and parental consent was granted.

Inulin clearance and urinary NGAL collection were performed the same day, between day 1 and 6 of life, in a population of preterm infants.

All preterm infants (gestational age less than 37 weeks) requiring a venous umbilical catheter were included. Exclusion criteria included severe anemia, hemodynamic instability and babies with birth weight smaller than 800 g.

Collected patient data included gestational age, birth weight, gender, and presence of IUGR. Exposition to renal insults as nephrotoxic medications, perinatal asphyxia, patent ductus arteriosus (PDA), and early onset sepsis were also recorded.

Urine was collected from the diaper using a cotton ball. The urine was then centrifuged and stored at −80 °C within 24 h after collection. The uNGAL ELISA was performed using a commercially available assay (Bioporto®, Hellerup, Denmark). The assay was performed as per the manufacturer's protocol.

The Inutest® (Sinistrine Fresenius Kabi, Linz, Austria) bolus injection and the collection of urine samples for the study were performed on the same day. Inulin determination at 30 min, 90 min, and 240 min after a single bolus injection of Sinistrine® (250 mg/kg over 3 min through the venous umbilical catheter, with a flush of NaCl) was performed using enzymatic assay. Inulin pharmacokinetics were analyzed using a standard non-compartmental analysis (NCA) model with WinNonlin software v. 6.2.1 (Pharsight, Mountain View, CA). The elimination rate constant (λ_z) was estimated from the slope of the terminal log-linear portion of the curve, and the terminal half-life ($t_{1/2}$) was calculated as $\ln 2/\lambda_z$. Clearance was calculated as follows: ($Cl = dose/AUC_{0-\infty}$).

Statistical analyses

Demographics will be analyzed by simple descriptive statistics. All continuous variables are expressed as means (standard deviation) or medians (interquartile interval) and categorical variables as frequencies. Normal distribution was assessed by an Anderson–Darling test. Due to the lack of Gaussian uNGAL distribution, non-parametric Mann–Whitney's tests were performed to compare median values between groups. The correlation between variables was assessed using Pearson's correlation coefficients tests after log transformation due to lack of Gaussian distribution. A two-sided p value$<.05$ was considered as statistically significant. Data were analyzed using STATA version 14.0 (StataCorp, College Station, TX).

Results

Study population

Forty-two preterm newborns were included in the study, 21 females. Four patients were extremely preterm infants (<28 weeks), 23 patients were very preterm infants (28–31 6/7 weeks), and 15 were late preterm (32–35 weeks). The mean gestational age was 30.7 ± 2.3 weeks and the mean birth weight was 1499 ± 430 g. Forty-five percent of our population has been exposed to a renal insult during the first five days of life (nephrotoxic medications, perinatal asphyxia, PDA, and early onset sepsis). Gentamicin is the most frequent one (15 patients) followed by early onset sepsis (four patients). Two patients with neonatal asphyxia and one with symptomatic PDA treated by indomethacin were included. Median study day was three days of life (range 1–6 days). The description of the study population is presented in Table 1.

The median uNGAL value was 122.8 ng/ml with a wide range of values between 7.1 and 1981.5 ng/ml (10^3 fold). Statistically significant higher uNGAL values were found in female newborns (188.7 ng/ml [36.8–1981.5] vs. 64.6 ng/ml [7–601]; p=.0005) (Table 2). uNGAL median values were decreasing between extremely preterm (<28 weeks), very preterm (28–32 weeks) and late preterm (32–35 weeks) groups (Table 2). There was a statistically significant inverse correlation between gestational age and uNGAL values (Pearson's coefficient $r = -.37$, p=.014). There was no correlation between birth weight and uNGAL values

(Pearson's coefficient $r = -=.12$, $p=.41$). uNGAL values were higher in intra-uterine growth restricted infants, in infants exposed to gentamicin and higher in infants exposed to neonatal asphyxia, early onset sepsis, and PDA treated by indomethacin without reaching statistical significance (Table 2).

Median inulin clearance was 18.8 ml/min/1.73 m^2 [14.8–25.5 ml/min/1.73 m^2] which is in accordance to the normal reference range for this preterm population in the first days of life. No patient in our population presented AKI. There is no correlation between uNGAL values and inulin clearance results (Pearson's coefficient $r=-.29$, p: .06).

Discussion

We described uNGAL values in a population of preterm newborns (mean gestational age 30 weeks) without AKI as confirmed by a gold standard technique (inulin clearance). We report an extremely wide range of uNGAL values, significant higher levels in females, and significant inverse correlation with gestational age. Higher uNGAL values were found when exposed to different perinatal renal insults such as gentamicin, perinatal asphyxia, early onset sepsis, and PDA treated by indomethacin.

Our median NGAL value was 122.8 ng/ml and is in accordance with other neonatal series. Lavery et al. [11] described a median level of uNGAL of 99.2 ng/ml in a series of 20 subjects with a birth weight less than 1500 g with a range of values as wide as in our series (0.51–2815.7 ng/ml, 10^3 fold). Suchojad et al. [14] described a median level of uNGAL of 144 ng/ml in a series of 57 preterm newborns with birth parameters comparable to our series (median gestational age of 30 weeks and mean birth weight of 1535 g). Our median value is much higher than in the Huynh series [17] (5 ng/ml) where 50 infants (mean gestational age: 29 weeks ± 1.6) with an uncomplicated neonatal course and without renal insults were selected.

Higher uNGAL values are found in female newborns as in others pediatric and adult series, with few explanations [11,14]. Huynh et al. [17] report possible contamination of female samples with neutrophils from vaginal secretions. Gender-specific uNGAL reference range values have to be established.

Correlation between gestational age and uNGAL has been described in other neonatal series. Huynh et al. [17] and Askenazi et al. [12] showed that uNGAL values are declining with gestational age by 20% with each weeks of life. Several hypotheses have been proposed to explain this relation: extreme preterm has higher uNGAL excretion due to tubular immaturity. Furthermore, the extreme preterm has a greater exposure to renal insults and comorbidities like sepsis

Table 1. Description of the study population.

Description of the study population	$N=42$	Mean	SD
Gender			
Female	21		
Male	21		
Gestational age (GA-weeks)			
		30.7	2.3
Extremely preterm (<28 GA)	4		
Very preterm (28–31 6/7GA)	23 (54.8%)		
Late preterm (≥32 GA)	15		
Birthweight (g)			
		1499	430
Renal insults			
One of the following renal insult	19 (45.2%)		
Gentamicin	15 (35.7%)		
Early onset sepsis	4		
Neonatal asphyxia	2		
PDA treated by indomethacin	1		

GA: gestational age.

Table 2. NGAL distribution.

NGAL distribution (ng/ml)	*N*	Median	Q25–Q75	Range
Population	42	122.8	60.5;225.9	7;1981.5
Female	21	188.7	118.3;672.6	36.8;1981.5
Male	21	64.6	29.1;127.1	7;601
Extremely preterm	4	812.2	207.2;1346.3	75.9;1453.9
Very preterm	23	124.4	64.6;223.3	31.4;1981.5
Late preterm	15	65.3	11.9;188.7	7.1;1091
IUGR	8	128	22.7;258.8	7.1;321.9
No IUGR	34	122.8	63.2;242.8	7.1;1981
Gentamicin +	15	151.9	70.6;377.8	11.9;1981.5
No gentamicin	27	115.2	46.7;171.5	7.1;1199.9
Early onset sepsis	4	144.3	65.1;224.2	64.6;226.8
No EOS	38	122.8	56;256.4	7;1981.5
Neonatal asphyxia	2	220.3		
No neonatal asphyxia	40	119	58.1;237	7;1981.5
PDA and indomethacin	1	129.9		
No PDA	41	121.8	59.2;226.8	7;1981.5

EOS: early onset sepsis.

that are described to influence and increase uNGAL levels. Moreover, *in vitro* studies suggested that uNGAL could take part in the nephrogenesis process and therefore higher in younger preterm infants [5,18].

Higher uNGAL values are found in infants group exposed to gentamicin. This finding is in accordance with other studies and may reflect an under-recognized tubular injury [19]. Jansen et al. [20] describe an increased urinary excretion of different biomarkers after gentamicin administration in a neonatal population. This rise precedes the peak of serum creatinine and the decrease in urinary output. Higher uNGAL values are found in infant groups exposed to neonatal sepsis or asphyxia and could also reflect undetected tubular injury. Unfortunately, this biomarker is also upregulated during inflammatory or ischemic processes. Several studies described a correlation between uNGAL values and the severity of sepsis and asphyxia, even in the absence of associated AKI. Studies also describe a positive correlation between uNGAL values and inflammatory markers like C-reactive protein and procalcitonin [14,21]. Currently, uNGAL production associated with tubular injury (as a monomer) or with inflammatory or ischemic processes (as a dimer) cannot be technically differentiated.

In our series, no preterm infants presented AKI diagnosis based on a reference method. Askenazi et al. [9] has described uNGAL in a series of 113 very low birth weight infants. Twenty-eight of them presented a neonatal AKI. It is the largest series studying uNGAL as an early AKI predictor in this population. The discriminative performance was moderate with an area under the curve of 0.67. The NGAL values in the AKI group were 1.8-fold higher than in the control group. Nevertheless, due to the wide range of values as described in our series, the overlap between two groups was high (623 ng/ml [343; 1670] vs. 349 ng/ml [204; 877]), limiting the usefulness and the interpretation of a single uNGAL value in the clinical setting.

In the specific setting of neonatal asphyxia, uNGAL performance to predict AKI has been more studied. However, the series mostly include term infants, without gestational age associated variations impact on uNGAL values. They described also wide range values and the proposed cut off values for the diagnosis of AKI across studies vary widely (18–652 ng/ml) [21].

The strength of this study is the description of uNGAL variations in a preterm population series without AKI as defined by a precise reference method and including a majority of very low birth weight infants. One limitation of our study is the limited number of renal insult such as asphyxia or PDA.

Conclusions

In this preterm newborn's series without AKI, the median uNGAL and its high variability are in accordance with published reference ranges. Correlation between uNGAL and gestational age exists, as well as gender impact. Newborns exposed to different renal insults in the neonatal period present higher uNGAL values, suggesting potential undetected tubular toxicity or reflecting NGAL production in case of inflammatory or ischemic processes. No correlation was found with inulin clearance.

Due to its high inter-individual variability and its lack of renal specificity, uNGAL usefulness and its discriminative capacity as a neonatal AKI biomarker has to be further determined in larger series.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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