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Effect of perinatal risk factors on neutrophil gelatinase-associated lipocalin (NGAL) level in umbilical and peripheral blood in neonates

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Abstract

Introduction: *Acute kidney injury biomarkers are opening a new era in diagnosing kidney failure. The requirement for a specific and sensitive marker of kidney function is highly desirable in neonates because the diagnostic possibilities in this age group are not sufficient. Recent research show that neutrophil gelatinase-associated lipocalin (NGAL) can have a great potential but there is a wide range of medical conditions, that may influence their expression.*

The aim of the study *was to evaluate the impact of perinatal risk factors on NGAL level in neonates.*

Material and methods: *NGAL was measured in umbilical cord blood and peripheral blood in full term neonates with perinatal risk factors during the first days of life.*

Results: *We found significantly higher umbilical cord blood NGAL levels in neonates with perinatal risk factors (117.69 ng/ml) compared to the control group (64.37 ng/ml). No significant difference in peripheral blood NGAL level was shown between the two groups. Umbilical cord blood NGAL level correlated positively with peripheral blood NGAL level ($r = 0.36$, $p < 0.01$). Umbilical cord blood NGAL level was significantly higher in neonates with fetal distress and infection compared to neonates with other perinatal risk factors. Peripheral blood NGAL level was significantly higher in neonates with infection compared to neonates with other perinatal risk factors. Significantly higher umbilical cord blood NGAL levels were seen in neonates born by operative delivery compared to born by natural delivery.*

Key words: *neonates, NGAL, AKI biomarkers.*

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Introduction

Neonates are the group that is most prone to acute kidney injury (AKI) [1, 2]. Chronic maternal disease and pregnancy- or birth-related pathology may have a negative impact on neonatal renal function. Risk factors for AKI in neonates include prematurity, intrauterine hypoxia, and hypovolaemia, e.g. due to sepsis [1, 3, 4]. A harmful effect on the development and function of neonatal kidneys is also exerted by non-steroidal anti-inflammatory drugs (NSAIDs), angiotensin-converting enzyme inhibitors (ACEI), and aminoglycoside antibiotics used in pregnant women or in the neonatal period [5].

The rate of AKI in neonates is 6-24% [2]. These figures are believed to be underestimated due to the (often) non-oliguric course of AKI and diagnostic challenges in this age group. Serum creatinine level remains the major diagnostic test for AKI, but it is a late marker of kidney damage [6]. Serum creatinine rises only two days after damage and/or with functional loss of >50% of renal parenchyma. In addition, during the first 48-72 hours of neonatal life, it reflects maternal creatinine level [7, 8].

Despite significant improvement in perinatal care, mortality due to AKI remains high, in the range of 10-61% [2]. Outcomes of AKI mostly depend on rapid treatment initiation, preferably already during the preclinical stage. Preliminary studies indicate the usefulness of new early biomarkers of AKI such as neutrophil-gelatinase-associated lipocalin (NGAL), kidney injury molecule 1 (KIM-1), and interleukin 18 [9-11].

Because the most common pathogenetic factors in neonatal AKI are hypovolemia and hypoxia, NGAL is believed to be the most promising marker. Its expression

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increases in the thick part of the ascending limb of loop of Henle and the collecting ducts in response to toxic agents or ischaemia [12].

An increasing spectrum of clinical conditions that were identified as being associated with increased NGAL expression makes it necessary to consider potential factors that might affect the specificity of the measurements and interpretation of the results. Until now, elevated serum and urine NGAL was noted in neonates with intrauterine hypoxia [9, 13, 14], sepsis [15], and bronchopulmonary dysplasia [16] and after cardiac surgery using extracorporeal membrane oxygenation [17]. In a group of neonates with intrauterine hypoxia, Essajee et al. found that urinary NGAL was a predictor or mortality and hypoxic encephalopathy [18]. Several authors noted a negative correlation between urinary NGAL level and birth weight and gestational age [8, 19-21]. Although data concerning umbilical cord blood NGAL concentration are rare, they reveal a usefulness of NGAL measurements as a marker of intrauterine hypoxia [22].

Until now no widely accepted reference NGAL levels in neonates have been established. Serum and urine NGAL levels differed between studies by different authors [20, 23]. Most available data were obtained in preterm infants.

The aim of the study was to evaluate the effect of selected perinatal risk factors on umbilical cord and peripheral blood NGAL levels in neonates during the first days of life.

Material and methods

The research was approved by Ethical Committee of the Medical University of Warsaw. All the parents signed the informed consent before samples were obtained from neonates.

The study included full-term neonates (gestational age ≥ 37 weeks) with potential perinatal risk factors (cases). The control group comprised 14 healthy full-term neonates.

Based on the available medical records, we analysed the course of pregnancy, taking into account maternal disease – hypertension, diabetes, infection during the last week of pregnancy, genital tract colonisation with *Streptococcus agalactiae* or *Escherichia coli* immediately before delivery, cholestasis of pregnancy, anaemia (haemoglobin level < 10 g/dl); medications used during pregnancy – antibiotics, NSAIDs, ACEI, steroid therapy; course of delivery – premature rupture of membranes (PROM) ≥ 6 hours (in accordance with local standards, women in labour are given antibiotic prophylaxis six hours after rupture of membranes), mode of delivery: spontaneous delivery or operative delivery (elective caesarean section, urgent caesarean section, vacuum-assisted delivery), foetal distress (as evidenced by decelerations in cardiotocography); neonatal condition after delivery - Apgar score at 1 and 5 minutes, birth weight, infection, congenital cardiac or renal disease, intrauterine hypoxia, and medications used.

In all neonates, NGAL level was measured in umbilical cord blood and peripheral blood serum obtained within five days of life (DOL 0-5). Serum was stored at –80°C until assays. Measurements were made by the immunoenzymatic method using NGAL ELISA Kit 036 (BIO Porto® Diagnostics), and the results were expressed in ng/ml.

A suspected infection was an indication for C-reactive protein (CRP) level measurement and/or complete blood count evaluation. Umbilical blood gases were indicated in cases of suspected neonatal hypoxia and in operative delivery.

Neonatal infection was diagnosed based on elevated CRP level and/or leukocyte count. Intrauterine hypoxia was defined as the Apgar score ≤ 6 at 1 minute and/or umbilical blood pH ≤ 7.1.

Statistical analysis

Statistical analysis was performed using Statistica 10 software (StatSoft). Normal distribution of the variables was tested using the Shapiro-Wilk test. Normally-distributed variables were expressed as mean values and standard deviation, and non-normally-distributed variables were expressed as median values and ranges. NGAL levels were log-transformed to obtain a normal distribution. Results were compared between groups using the Student t test, and relations between the evaluated variables were assessed using linear regression. $P < 0.05$ was considered statistically significant.

Results

We studied 62 neonates (33 males and 29 females) with perinatal risk factors at a mean gestational age of 38.9 ±0.92 weeks, and 14 healthy neonates (nine males and five females) at a mean gestational age of 38.61 ±1.27 weeks, who constituted the control group. Clinical characteristics and details regarding the mode of delivery are shown in Table 1.

We found no significant differences between the study and control groups with regard to gender distribution, gestational age, birth weight, and Apgar score at one and five minutes (Table 1).

In 11% of women in the study group and in one (7%) woman in the control group the risk of premature delivery was diagnosed. All these women were treated using glucocorticosteroids.

Antibiotics (ampicillin, amoxicillin-clavulanate, or cefuroxime) due to PROM ≥ 6 hours, genital tract colonisation, or infection were used in 42 women. No women required treatment with NSAIDs or ACEI.

Antibiotic therapy with ampicillin and netilmicin was used due to intrauterine infection in seven neonates (11%).

Table 1. Clinical characteristics of neonates with potential risk factors for acute kidney injury (AKI)

	Cases (n = 62)	Controls (n = 14)	p
Gender, n (%)			
Male	33 (53)	9 (64)	NS
Female	29 (47)	5 (36)	NS
Gestational age (weeks)	38.93 ±0.92	38.61 ±1.27	NS
Mode of delivery, n (%)			
Vaginal	25 (41)	9 (64)	NS
Operative**	37 (59)	5 (36)	NS
Birth weight (g)	3325.00 ±442.31	3312.74 ±488.95	NS
Apgar score*			
1-min	10 (5-10)	10 (8-10)	NS
5-min	10 (6-10)	10 (8-10)	NS

**Median (range).*
***Caesarean section and vacuum extractor*

Table 2. Umbilical cord and peripheral blood NGAL levels (median, range) in full-term neonates with perinatal risk factors and the control group.

	NGAL (ng/ml)		p
	Cases (n = 62)	Controls (n = 14)	
Umbilical cord blood NGAL	117.69 (17.47-536.57)	64.37 (30.36-142.04)	< 0.01
Peripheral blood NGAL	179.11 (18.10-571.93)	155.28 (34.07-445.57)	NS
	$p < 0.05$	$p < 0.05$	

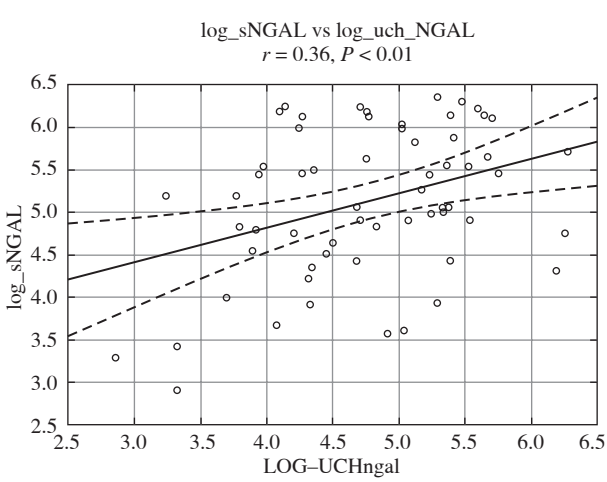

Fig. 1. Correlation between log-transformed umbilical cord NGAL (log_ucbNGAL) and peripheral blood NGAL (log_sNGAL) levels in neonates with perinatal risk factors

Potential risk factors for kidney injury were divided into three categories: maternal disease and pregnancy-related, perinatal, and neonatal. Chronic maternal disease or pregnancy-related pathology was found in 51 pregnant women (82%), most commonly diabetes in 23 (37%) and genital tract colonisation with *Streptococcus agalactiae* or *Escherichia coli* before the delivery in 22 (35%). Infection was diagnosed in 20 pregnant women (32%), including elevated CRP level (median 29 mg/dl, range 12-190 mg/dl) in 17, and leukocyte count > 16,000/μl in the remaining three in whom CRP level was not measured. Hypertension was diagnosed in seven mothers (11%), anaemia with haemoglobin level < 10 g/dl in three (6%), and intrahepatic cholestasis of pregnancy in two (3%).

Perinatal risk factors were noted in 41 neonates (66%). PROM and foetal distress were found in 26 cases (42%), and both these factors were present in 11 of these children.

Neonatal risk factors were present in 26 children (42%): congenital defects in 17 (27%), including cardiac in 11 neonates and urinary in six neonates; and infection in 10 neonates (16%), with the diagnosis based on an elevated CRP level (mean 23.5 ±7.71 mg/dl) with the mean leukocyte count of 20,300 ±10,480/μl in nine neonates, and based on isolated leucocytosis (40,000/μl) in one neonate; intrauterine hypoxia was found in four neonates, with the diagnosis based on low umbilical blood pH of 7.04-7.1 in three neonates; two neonates had low Apgar score at one minute (five and six points, respectively).

Overall, one to seven perinatal risk factors were present in individual neonates.

Umbilical cord and peripheral blood NGAL levels are summarised in Table 2. Both umbilical cord and peripheral blood NGAL level in neonates with perinatal risk factors was higher compared to the control group, although statistical significance was reached only for umbilical cord blood NGAL level ($p < 0.01$). Both in the study group and the control group, NGAL level was significantly higher in peripheral blood than in umbilical cord blood ($p < 0.05$). The mean timing of peripheral blood collection in the study group and the control group was similar (1.78 ±0.93 and 1.49 ±0.83 DOL, respectively). We found a significant positive correlation between log-transformed umbilical cord and peripheral blood NGAL levels (r = 0.36; $p < 0.01$) (Fig. 1).

The effect of specific perinatal risk factors on umbilical cord and peripheral blood NGAL levels is shown in Table 3 and 4. The highest umbilical cord blood NGAL levels were noted in neonates with infection and intrauterine hypoxia. Umbilical cord blood NGAL levels in neonates with perinatal risk factors related to pregnancy-related pathology and maternal disease (diabetes, maternal infection, genital tract colonisation, hypertension), perinatal pathology (PROM, foetal distress), and neonatal pathology (infection, congenital defects) were significantly higher ($p < 0.05$) compared to the control group. Due to low sample

Table 3. Comparison of umbilical cord blood NGAL levels in neonates with a specific risk factor vs. neonates with other risk factors and the control group

Risk factor	Neonates with a given risk factor present		Neonates with other risk factors		Control group		p^1	p^2
	n	NGAL (ng/ml)	*n*	NGAL (ng/ml)	*n*	NGAL (ng/ml)		
Maternal disease / pregnancy-related pathology								
Diabetes	23	75.24 (25.57-302.97)	39	126.47 (17.47-536.57)	14	64.34 (30.36-42.04)	NS	< 0.05
Infection	20	143.38 (25.57-536.57)	42	114.02 (17.47-519.80)			NS	< 0.001
Genital tract colonisation	22	132.92 (27.82-519.80)	40	117.69 (17.47-536.57)			NS	< 0.01
Hypertension	7	90.99 (58.89-209.61)	55	126.47 (17.47-536.57)			NS	< 0.05
Other	5	168.21 (76.41-219.03)	57	116.23 (17.47-536.57)			NS	< 0.005
Perinatal pathology								
PROM	26	110.12 (17.47-536.57)	36	139.53 (27.82-519.80)			NS	< 0.05
Foetal distress	26	122.30 (27.82-491.94)	36	101.40 (17.47-536.57)			< 0.05	< 0.01
Neonatal pathology								
Early-onset infection	10	209.69 (43.46-536.57)	52	116.73 (17.47-491.94)			< 0.05	< 0.001
Congenital malformation	17	126.47 (17.47-282.52)	45	116.23 (25.57-536.57)			NS	< 0.05
Intrauterine hypoxia	4	197.17 (17.47-491.94)	58	117.69 (25.57-536.57)			–	–

PROM – premature rupture of membranes
p^1 – neonates with a given risk factor vs. neonates with other risk factors
p^2 – neonates with a given risk factor vs. control group

size, neonates with intrauterine hypoxia were excluded from statistical analysis. Umbilical cord blood NGAL levels were significantly higher in neonates with infection and foetal distress compared to neonates with other perinatal risk factors ($p < 0.05$).

Similarly to umbilical cord blood, the highest peripheral blood NGAL levels were observed in neonates with infection and intrauterine hypoxia. In neonates with infection, peripheral blood NGAL level was significantly higher compared to neonates with other risk factors. Regardless of the analysed risk factor, we found no statistically significant differences in peripheral blood NGAL level compared to the control group.

Both umbilical cord and peripheral blood NGAL level did not increase with increasing number of perinatal risk factors present.

Evaluation of the effect of mode of delivery on NGAL levels in neonates showed significantly higher umbilical cord blood NGAL levels in neonates born by operative delivery compared to those born by natural delivery (Table 5). Mode of delivery also had no effect on peripheral blood NGAL levels.

Discussion

Major advances that have occurred in the recent years in perinatology have not translated into improved ability to detect kidney injury in neonates. Due to limitations of the diagnosis of AKI based on serum creatinine level, increasing attention has been paid to new early AKI biomarkers that allow more rapid and specific identification of patients with kidney damage in older age groups.

In our study, umbilical cord blood NGAL levels in the control group (64.37; 30.36-42.04 ng/ml) were similar to peripheral blood NGAL levels at 1 DOL in healthy neonates reported by other authors [14, 24].

Due to an increasing spectrum of clinical conditions, in addition to kidney injury, in which elevated NGAL levels are seen in body fluids [25-27], in the present study we evaluated the effect of perinatal risk factors on umbilical

Table 4. Comparison of peripheral blood NGAL levels in neonates with a specific risk factor vs. neonates with other risk factors and the control group

Risk factor	Neonates with a given risk factor present		Neonates with other risk factors		Control group		*p*1	*p*2
	n	NGAL (ng/ml)	*n*	NGAL (ng/ml)	*n*	NGAL (ng/ml)		
Maternal disease / pregnancy-related pathology								
Diabetes	23	178.60 (18.10-509.06)	39	193.86 (26.78-571.93)	14	155.28 (34.07-445.57)	NS	NS
Infection	20	153.39 (53.48-542.81)	42	210.78 (18.10-571.93)			NS	NS
Genital tract colonisation	22	241.14 (30.61-571.93)	40	156.37 (18.10-509.06)			NS	NS
Hypertension	7	103.27 (38.86-277.45)	55	193.86 (18.10-571.93)			NS	NS
Other	5	83.65 (49.78-336.28)	57	193.86 (18.10-571.93)			NS	NS
Perinatal pathology								
PROM	26	237.97 (18.10-507.83)	36	156.37 (30.61-571.93)			NS	NS
Foetal distress	26	175.81 (30.61-542.81)	36	179.11 (18.10-571.93)			NS	NS
Neonatal pathology								
Early-onset infection	10	457.35 (76.87-571.93)	52	156.37 (18.10-509.06)			< 0.05	NS
Congenital defects	17	135.39 (26.78-467.92)	45	227.71 (18.10-571.93)			NS	NS
Intrauterine hypoxia	4	269.61 (26.78-507.83)	58	179.11 (18.10-571.93)			---	---

PROM – premature rupture of membranes
p^1 – neonates with a given risk factor vs. neonates with other risk factors
p^2 – neonates with a given risk factor vs. control group

Table 5. Umbilical cord and peripheral blood NGAL levels (median, range) in neonates with perinatal risk factors in relation to the mode of delivery

NGAL (ng/ml)	Mode of delivery		*p*
	Operative (*n* = 37)	Natural (*n* = 25)	
Umbilical cord blood	168.21 (27.82-536.57)	90.99 (17.47-519.80)	< 0.05
Peripheral blood	178.60 (30.61-571.93)	179.61 (18.10-509.06)	NS

cord and peripheral blood NGAL level during the first five days of life.

We found a strong effect of infectious factors on NGAL levels in the study group, which may be explained by NGAL involvement in antibacterial defence mechanisms. Previous studies showed increased NGAL levels in body fluids, e.g. in urinary tract infections [28] and sepsis [29]. Tadesse *et al*. showed increased trophoblast NGAL expression in intrauterine infections [24]. In our study group, we found significantly higher NGAL levels in both umbilical cord blood and peripheral blood in neonates with infection. Similarly, albeit insignificantly, increased umbilical cord and peripheral blood NGAL levels were seen in neonates born by mothers with an infection during the last week of pregnancy. These findings indicate that inflammation is one of the factors that exert an effect on NGAL levels in body fluids independently of renal function, and this effect should be taken into account when NGAL level is used as a biomarker of AKI. The finding of higher umbilical cord blood NGAL levels indicates that it may be a potential early marker of neonatal inflammation.

Another evaluated factor that is of importance for increased NGAL expression is hypoxia, considered a major cause of AKI in neonates. Although identification of intrauterine hypoxia in only four neonates precluded statistical

analysis, both umbilical cord and peripheral blood NGAL levels in these neonates were higher compared to neonates born in a general good condition. In our study group, significantly higher umbilical cord blood NGAL levels were seen in neonates with foetal distress. These results are in accordance with observations of other authors who showed elevated NGAL levels in neonates with asphyxia, both with AKI and with normal renal function, measured in umbilical cord blood [22] and peripheral blood [9, 13, 14].

Although increased umbilical cord blood NGAL levels compared to the control group were seen in neonates at risk due to maternal disease such as diabetes, infection, or hypertension, perinatal pathology such as PROM, or congenital defects, the differences were not significant in comparison to other perinatal risk factors.

It is unclear why significantly higher umbilical cord blood NGAL levels were found in 37 neonates (60%) born by operative delivery compared to those born by normal delivery. Although it might have been related to higher rates of risk factors in this group, significantly higher umbilical cord blood NGAL levels were also seen in the control group where the decision to perform a caesarean section was due to maternal indications (e.g. maternal visual impairment or previous caesarean section). Understanding of the effect of mode of delivery on NGAL levels requires further studies. No effect of mode of delivery on peripheral blood NGAL level may suggest that its source was the placenta and not kidneys of the neonate.

Our findings indicate that neonatal infection and intrauterine hypoxia or mode of delivery may affect NGAL levels in neonatal body fluids during the first days of life, a fact that should be taken into account when interpreting NGAL level measurement results. Definite determination of the effect of specific perinatal risk factors on neonatal NGAL levels would require a larger study, allowing multivariate analysis or identification of a larger number of neonates with isolated specific risk factors.

Conclusions

1. Neonatal NGAL levels are affected by infection and intrauterine hypoxia.

2. NGAL level in umbilical cord blood may be an early noninvasive marker of neonatal infection.

The authors declare no conflict of interest.

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