Umbilical cord blood NGAL concentration as an early marker of perinatal asphyxia in neonates

Stężenie NGAL w krwi pępowinowej jako wczesny marker niedotlenienia okołoporodowego u noworodków

Piotr Surmiak, Małgorzata Baumert, Małgorzata Fiala, Kinga Sypniewska, Zofia Walencka, Agnieszka Łukomska, Karolina Karcz

Klinika Neonatologii Katedry Położnictwa i Ginekologii, Śląski Uniwersytet Medyczny, Katowice, Polska

Abstract

Introduction: *Recent reports have revealed increased concentration of neutrophil gelatinase-associated lipocalin (NGAL) in cardiovascular diseases and after episodes of hypoxia. We hypothesized that elevated plasma NGAL levels could be a result of vascular endothelial injury due to perinatal asphyxia.*

Materials and methods: *Ninety-three newborns with a gestational age ⩾37 weeks, of which 32 newborns were asphyxiated (study group), and 61 were healthy children (control group), were enrolled in the study. Serum NGAL, lactate and creatinine concentrations, acid-base balance, neutrophil and white blood cell count were measured in the umbilical cord blood.*

Results: *Asphyxiated newborns had a significantly lower pH value (7.0 vs. 7.3; p<0.001), lower HCO3 (15.8mmol/L vs. 23.2mmol/L; p<0.001) and higher lactate concentrations (7.5mmol/L vs. 2.3mmol/L; p<0.001), as compared to controls. Neutrophil count (10.3x109/L vs. 6.5x109/L; p=0.02) and NGAL concentration (122.5ng/mL vs. 24.3ng/mL p<0.001) were elevated in asphyxiated newborns as compared to healthy children.*

Conclusions: *The measurement of NGAL in the umbilical blood can be a valuable biomarker of perinatal asphyxia in neonates.*

Key words: **newborn** / **asphyxia** / **NGAL** /

Streszczenie

Wstęp: *Badania z ostatnich lat wykazały wzrost stężenia lipokainy związanej z żelatynazą neutrofilii (NGAL) w chorobach sercowo-naczyniowych i po epizodach niedotlenienia. Chcielibyśmy sprawdzić, czy zwiększone stężenie NGAL w surowicy może być wynikiem uszkodzenia śródbłonka naczyń w wyniku niedotlenienia okołoporodowego.*

Materiał i metoda: *Do badań zakwalifikowano 93 noworodki w wieku płodowym ⩾37 tygodni ciąży, w tym 32 z objawami niedotlenienia i 61 zdrowych dzieci jako grupa kontrolna. W surowicy krwi pępowinowej oznaczano stężenia NGAL, laktatów, kreatyniny, równowagę kwasowo-zasadową oraz liczbę neutrofili i krwinek białych.*

Corresponding author:
Piotr Surmiak
Klinika Neonatologii SP CSK im. Prof. Gibińskiego
ul. Medyków 14, 40-752 Katowice, Polska
tel.: +48 509 792 558
e-mail: piotrek.surmiak@gmail.com

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Wyniki: *Noworodki z objawami niedotlenienia miały znamiennie niższą wartość pH (7,0 vs 7,3; p<0,001), niższe stężenie HCO3 (15,8mmol/L vs 23,2mmol/L; p<0,001) i wyższe stężenie mleczanów (7.5mmol/L vs 2.3mmol/L; p<0,001) w porównaniu z grupą kontrolną. Liczba neutrofili (10,3x109/L vs 6,5x109/L; p=0,02) i stężenie NGAL (122,5ng/mL vs 24,3ng/mL; p<0,001) było znacząco wyższe u noworodków z grupy niedotlenionych w porównaniu z grupą kontrolną.*

Wnioski: *Oznaczanie NGAL z krwi pępowinowej może być ważnym markerem w diagnostyce niedotlenienia okołoporodowego.*

Słowa kluczowe: **noworodek** / **niedotlenienie** / **NGAL** /

Introduction

Perinatal asphyxia, estimated to occur in 2-6/1000 live-born infants, constitutes one of the most severe complications of contemporary neonatology [1, 2]. Its incidence has been increasing in the developing countries [3]. Perinatal asphyxia creates a significant reason for morbidity and mortality in the first days of neonatal life and is also the cause of multiorgan complications, with damage to the central nervous system, kidneys, and cardiovascular system, among the most severe examples [4]. Continuous research is being conducted to discover early biomarkers of perinatal asphyxia, thus allowing prompt diagnosis and treatment commencement to prevent further complications.

Lately, much attention has been drawn to neutrophil gelatinase-associated lipocalin (NGAL) – a small particle, approximately 25kDa in size, resistant to degradation [5].

Recent studies have revealed a significant increase in NGAL concentration, both in serum and urine, already after a mere few hours since the occurrence of hypoxia, and the results persisted for some days thereafter [6, 7, 8]. We hypothesized that elevations of plasma NGAL could be caused by an injury to the endothelial cells in vessels due to perinatal asphyxia. The aforementioned reports allowed us to initiate an investigation about the relevance of the umbilical NGAL concentration as a marker of perinatal asphyxia.

Materials and methods

Newborn babies with completed gestational age of 37 weeks, admitted to the Department of Neonatology, Medical University of Silesia, Katowice, Poland, between November 1, 2012 and April 30, 2013, were deemed eligible for the study if cord blood serum had been drawn and stored immediately after delivery. Exclusion criteria for the investigation were: congenital malformations, inborn errors of metabolism, intrauterine growth retardation, systemic infection or blood group incompatibility, and maternal hypertension, diabetes, liver and kidney diseases.

Acute perinatal asphyxia was defined, when two of the following were presented: history of fetal distress (bradycardia <100 beats/min, late decelerations for >10 min. and/or a silent pattern on cardiotocography); Apgar of ≤6 at 5 minutes and the need for resuscitation for >1 min. with positive-pressure ventilation and oxygen after birth; pH value ≤7.20, and base deficit >12 mmol/L in the umbilical artery.

Ninety-three infants were enrolled in the study. Based on the aforementioned criteria, 32 (34%) newborns, diagnosed with perinatal asphyxia, constituted the study group and 6 (20%) of them developed acute kidney injury (AKI). AKI was defined as an elevation of serum creatinine ≥1.5 mg/dL in the second day of life [9]. The control group consisted of 61 healthy neonates matched for gestational age, birth weight and postnatal age.

The study was approved by the Human Ethics Committee, Medical University of Silesia, and a written parental consent was obtained before enrollment of each infant.

Laboratory investigations included neutrophil and white blood cell (WBC) counts, arterial blood gas analyses, lactate and creatinine levels (from the umbilical artery and daily from the peripheral vessels of each newborn for the first two days of neonatal life), and serum NGAL (from the umbilical artery after delivery).

NGAL cord blood concentrations were determined using the sandwich enzyme immunoassay for the quantitative measurement of human lipocalin-2 (BioVendor – Laboratorní medicína a.s., Brno, Czech Republic). The detection limit of the assay was 0.02 ng/mL. Blood gas analyses were established using a model Rapidlab 865 Blood Gas Analyzer (Siemens Medical Solutions Diagnostics, Bad Nauheim, Germany).

Statistical analysis

Statistical analysis was performed using standard procedures available in STATISTICA 10 (Statsoft Polska Inc.). The normal distribution was tested using the Shapiro-Wilk test, while statistical significance differentiating the two groups were assessed using the Mann-Whitney U test and amongst three groups, by performing the nonparametric ANOVA (Kruskal-Wallis test). Quantitative variables are presented as median, minimum and maximum values, whereas, qualitative variables are presented as percentages. A correlation study between different analyzed parameters was performed using the Spearman's rank correlation coefficient test for skewed data. The statistical inferences were based on the level of significance $p<0.05$.

Results

Table I presents the demographic and perinatal data of all 32 asphyxiated neonates and the control group. No differences were observed with reference to the gestational age, gender, birth weight and mode of delivery between asphyxiated and non-asphyxiated infants. However, asphyxiated neonates were born in a worse general condition according to the Apgar score at 5 minutes, as compared to controls (5 vs. 8 points; $p<0.001$). We observed a lower pH value (7.0 vs. 7.3; $p<0.001$), lower HCO3-

Table I. Characteristics of newborns enrolled in the study.

Variable	Asphyxiated group n=32		Control group n=61		
	Me	(min./max.)	Me	(min./max.)	p*
WG [wk]	37.9	(37.0; 40.0)	38.2	(37.0; 41.0)	NS
Birthweight [g]	2914	(2055; 4300)	3141	(2250; 4440)	NS
Apgar 5th min. [pts]	5	(0; 7)	8	(6; 10)	<0.001
Gender f/m [%]	50/50		48/52		NS
Hb [g/dL]	16.1	(13.6; 18.3)	15.1	(9.2; 19.2)	NS
Neutrophils [10^9/L]	10.3	(1.1; 19.4)	6.5	(2.9; 12.5)	0.02
WBC [10^9/L]	17.7	(5.4; 18.5)	15.4	(6.5; 17.3)	NS
HCO3 [mmol/L]	15.8	(8.0; 23.8)	23.3	(17.9; 29.7)	<0.001
Lactate [mmol/L]	7.5	(2.6; 17.7)	2.3	(1.0; 6.5)	<0.001
pH	7.0	(6.8; 7.2)	7.3	(7.2; 7.4)	<0.001
Serum creatinine [mg/dL]	0.9	(0.4; 1.5)	0.7	(0.4; 1.2)	<0.001
Serum NGAL [ng/mL]	122.5	(38.5; 273.0)	24.3	(3.3; 88.3)	<0.001

Me – Median; **WG** – weeks of gestation; **Hb** – hemoglobin; **WBC** – white blood cells; **NGAL** – neutrophil gelatinase-associated lipocalin.; **p*** – from Mann-Whitney U test

Table II. NGAL concentration in the study and the control groups.

	NGAL			
	N	Me	(min./max.)	p*
(1) pH < 7.0	23	154.5	(60.5; 273.0)	(1) vs (2)
(2) pH 7.1-7.2	13	89.4	(38.5; 180.6)	(2) vs (3)
(3) pH >7.2	57	31.5	(11.7; 130.0)	(3) vs (1)

Me – Median; **NGAL** – neutrophil gelatinase-associated lipocalin.; **p*** <0.05 – ANOVA (Kruskal-Wallis test)

(15.8mmol/L vs. 23.2mmol/L; p<0.001), and higher lactate concentrations (7.5mmol/L vs. 2.3mmol/L; p<0.001) in the study group as compared to the control group. Neutrophil count was significantly elevated in asphyxiated children in comparison to healthy controls ($10.3x10^9$ /L vs. $6.5x10^9$ /L; p=0.02). There were no observable differences in the hemoglobin level (16.1g/dL vs. 15.1g/dL; p>0.05), and white blood cell (WBC) count ($17.7x10^9$/L vs. $15.4x10^9$/L), between newborns from the study group and controls.

We noted an elevated NGAL concentration (122.5ng/mL vs. 24.3ng/mL p<0.001) and creatinine level (0.9mg/dL vs. 0.7mg/dL; p<0.001) in the asphyxiated group as compared to the control group. Furthermore, we also observed increased NGAL and serum creatinine levels (≥ 1.5 mg/dL) in 6 newborns suffering from AKI from the study group in comparison to asphyxiated non-AKI infants (122.5ng/mL vs. 176.3ng/mL; p=0.05) and controls (176.3ng/mL vs. 24.3ng/mL; p<0.001) – Figure 1.

We noticed an inverse correlation between the concentration of NGAL and the general well-being of neonates assessed according to the Apgar score at 5 minutes (r=-0.37; p<0.05), and pH value of the umbilical blood (r=-0.55; p<0.05) (Table II), while a positive correlation between NGAL and lactate concentrations of the umbilical blood (r=0.57; p<0.05), as well as the neutrophil count therein (r=0.15; p<0.05), was found.

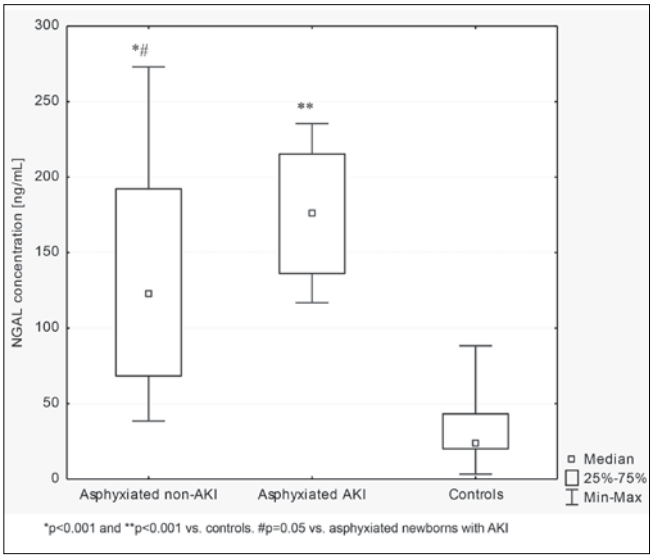

Figure 1. RUmbilical cord serum NGAL concentration in asphyxiated AKI and non-AKI neonates, and controls.

Discussion

We compared the serum level of NGAL in neonates with symptoms of acute perinatal asphyxia to healthy controls. According to our results, asphyxiated neonates, especially those that suffered from AKI, had significantly increased levels of NGAL, in comparison to those that were non-asphyxiated. Similar results had been attained by Raggal NM et al., and Sarafidis et al., [6, 8].

Additionally, we established that the concentration of NGAL correlated with low Apgar score at 5 minutes, and increasing metabolic acidosis, suggesting that NGAL could possibly be a valuable indicator of perinatal asphyxia.

Neutrophil gelatinase-associated lipocalin, known as the innate immunity antibacterial factor, is released in insignificant amounts by activated neutrophils in several human tissues. This factor is grossly induced as a response to a variety of injuries to epithelial and renal tubular cells [10, 11, 12].

Asphyxia, including perinatal asphyxia, is known to causes damage to the walls of blood vessels. This has been proven in studies by several authors, for example Nako et al., and Buchner et al., [13, 14]. Vascular wall damage provides a significant source of NGAL in serum through the activation of neutrophils. Some authors suggest its role in the inflammatory reaction, following an incidence of asphyxia and vascular wall remodeling due to injury, as the reason for enhanced NGAL expression from activated neutrophils [6, 15].

In our study, we also observed an increased value of neutrophils in asphyxiated infants, especially those who developed AKI, in comparison with the control group, and the asphyxiated group without AKI. Perhaps the damage to the vascular wall and developing inflammatory process therein activated neutrophils, which are the source of NGAL. This can be confirmed by the positive correlation obtained between the number of neutrophils and NGAL concentration. Inflammation as a result of asphyxia cannot be ruled out in our study.

Endothelial injury may lead to multiorgan damage, with the most severe complications affecting the central nervous system, cardiovascular system, and kidneys [16].

Most recent studies have suggested that NGAL may be a sensitive and early biomarker of AKI, detected both in serum and urine of patients in just a few hours after the damaging factor had been activated, which is in contrast to creatinine, whose increased concentration only appears after 24-48 hours, resulting in delayed diagnosis and treatment. In case of asphyxia in neonates, determining the degree of kidney function on the base of creatinine concentration is unreliable, as serum creatinine level in newborns in the first few days of neonatal life reflects maternal renal function due to placental transfer [17, 18, 19].

A small number of asphyxiated neonates enrolled into the study group was the main limitation of our investigation.

Conclusion

In conclusion, asphyxiated neonates demonstrate significantly increased NGAL levels in comparison to healthy controls, and the measurement of this biomarker in umbilical blood following acute asphyxia can be of significant diagnostic value. Due to the positive correlation demonstrated between NGAL and the Apgar score, as well as umbilical pH value, it seems safe to assume that NGAL could serve as a valuable marker of asphyxia in neonates.

Oświadczenie autorów

1. Piotr Surmiak – autor założeń pracy, analiza statystyczna wyników, przygotowanie manuskryptu i piśmiennictwa – autor zgłaszający i odpowiedzialny za manuskrypt.
2. Małgorzata Baumert – autor koncepcji i założeń pracy, analizy i interpretacji wyników, przygotowanie, korekta i akceptacja ostatecznego kształtu manuskryptu.
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5. Zofia Walencka – uzyskanie funduszy na realizację badań laboratoryjnych, autor koncepcji i założeń pracy, analizy i interpretacji wyników, ostateczna weryfikacja i akceptacja manuskryptu.
6. Agnieszka Łukomska – opracowanie koncepcji i założeń pracy, zbieranie materiału, opracowanie wyników badań.
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