Umbilical markers of perinatal hypoxia

Markery niedotlenienia okołoporodowego w krwi pępowinowej

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Abstract

Objectives: *The aim of the study was to evaluate activin A and NGAL levels as potential early markers of perinatal hypoxia.*

Material and methods: *We prospectively studied 58 full-term newborns: 24 with perinatal hypoxia (study group) and 34 healthy controls. Umbilical cord blood samples were obtained from all subjects immediately after delivery for the measurement of activin A and NGAL levels. Both biomarkers were correlated with biochemical indicators od hypoxia and neonatal complications.*

Results: *Activin A levels were significantly higher in hypoxic as compared to non-hypoxic newborns (0.51 vs. 0.22pg/mL; p<0.01). NGAL levels were also higher in asphyxiated babies as compared to controls (99.1 vs. 22.3ng/mL; p<0.001). A correlation between NGAL and activin A levels was detected (R=0.54; p<0.01). NGAL concentration was also correlated with Apgar score at 5 min. and pH value, HCO3, based deficit and lactate levels. ROC curve analysis demonstrated the cutoff value of >33.9ng/ml for NGAL in prediction of perinatal asphyxia in neonates, with a sensitivity of 100% and specificity 78.3%, whereas the cutoff value for activin A was 0.208ng/ml had, with a sensitivity of 93.1% and only 26.7% specificity.*

Conclusions: *Asphyxiated neonates demonstrate elevated NGAL and activin A levels as compared to controls. The correlation of NGAL with clinical and biochemical signs of neonatal hypoxia, as well as higher sensitivity and specificity for NGAL measurements, have led us to believe that NGAL could be a better marker of perinatal hypoxia than activin A.*

Key words: **perinatal hypoxia** / **markers** / **neonatal complications** / **newborns** /

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Streszczenie

Cel pracy: Ocena przydatności oznaczenia aktywiny A oraz NGAL, jako wczesnych markerów niedotlenienia okołoporodowego.

Materiał i metody: Prospektywnym badaniem objęto 58 donoszonych noworodków. Grupę badaną stanowiły 24 noworodki z niedotlenieniem okołoporodowym, a 34 zdrowe dzieci - grupę kontrolną. Uzyskano próbki krwi pępowinowej od wszystkich uczestników, natychmiast po odpępnieniu, w celu oznaczenia stężenia aktywiny A oraz NGAL. Badano związek pomiędzy stężeniem markerów a biochemicznymi wykładnikami niedotlenienia i powikłaniami u noworodków.

Wyniki: Stężenie aktywiny A było znamiennie wyższe u niedotlenionych noworodków w porównaniu z dziećmi zdrowymi (0,51 vs 0,22pg/mL; p<0,01). Zauważono także wyższe stężenie NGAL w grupie niedotlenionych dzieci w porównaniu z kontrolą (99,1 vs 22,3ng/mL; p<0,001). Stwierdzono związek pomiędzy stężeniem NGAL i aktywiny A (R=0,54; p<0,01) oraz stężeniem NGAL a punktacją w skali Apgar w 5 min. życia, wartością pH, stężeniem HCO3, niedoboru zasad i laktatów. Analiza krzywej ROC wykazała czułość 100% i specyficzność 78,3% dla stężenia NGAL >33,9ng/mL w predykcji niedotlenienia okołoporodowego. Natomiast dla wartości stężenia aktywiny A >0,208ng/mL czułość wynosiła 93,1% a specyficzność 26,7%.

Wnioski: U niedotlenionych noworodków stwierdzono wyższe stężenie NGAL i aktywiny A w porównaniu do kontroli. Wykazany związek pomiędzy stężeniem NGAL a klinicznymi i biochemicznymi wykładnikami niedotlenienia, a także wyższa czułość i specyficzność dla oznaczenia stężenia NGAL, sugerują że NGAL może być lepszym markerem niedotlenienia okołoporodowego niż stężenie aktywiny A.

Słowa kluczowe: **markery niedotleniowe dookołoporodowe** / **noworodki** /
/ **krew pępowinowa** / **powikłania** /

Introduction

Perinatal hypoxia (PH) is a medical condition which involves impaired blood gas exchange during the intrapartum period [1]. The prevalence of perinatal hypoxia is estimated at 3.0 per 1000 live births [2]. It has been widely accepted that severe neonatal encephalopathy following PH can lead to serious motor disabilities, mental retardation, and seizure disorders [3]. Neonatal mortality attributable to birth asphyxia is estimated at 25% within the first month of neonatal life [4]. Approximately 25% of the surviving newborns exhibit permanent neuropsychological deficits [5].

Perinatal hypoxia may lead to multiorgan damage with the most severe complications affecting the central nervous system, cardiovascular system, and kidneys. The developing fetal brain is highly dependent on sustained blood flow, due to the absence of its own energy and nutrient reserves. The severity, intensity and timing of asphyxia, as well as selective ischemic vulnerability and the immaturity of the brain, determine the extension and degree of severity of the ensuing damage. In addition to the irreversible lesion which occurs during ischemia, a significant portion of cellular death occurs after an ischemic insult to the brain. Therefore, treatment strategies should be developed to reduce the magnitude of cerebral injury following asphyxia by blocking the cytotoxic mechanisms which occur during the post-ischemic, reperfusion phase of recovery [6].

On the basis of the aforementioned data, the key word is prevention, with the aim of improving our ability to detect the fetuses and newborns at risk of brain injury at the earliest stage, when the window for therapeutic action is still open (the first 6h after hypoxia).

One of such potential biochemical markers of perinatal hypoxic brain damage is activin A. Activin A is a dimeric glycoprotein composed of 2 βA subunits which belongs to the transforming growth factor-β superfamily of differentiation factors [7]. Studies conducted on experimental models, as well as in humans with acute brain injury, strongly present enhanced activin A expression as a common response to acute neuronal damage of various origins [8]. Hypoxic/ischemic injury, mechanical irritation, and chemical damage of the brain evoke a strong upregulation of activin A [9].

Another promising marker of perinatal hypoxia is neutrophil gelatinase-associated lipocalin (NGAL). Several studies performed in the last couple of years revealed a significant increase in NGAL concentration, both in serum and urine, just a few hours after which hypoxia had developed, and these results persisted for a few days thereafter [10,11,12]. NGAL, known as the innate immunity antibacterial factor, is a 25 kDa secretory glycoprotein belonging to the lipocalin family of proteins [13]. This factor is grossly induced as a response to a variety of epithelial injuries [14,15,16]. Asphyxia, in which perinatal asphyxia is also included, has been known to cause damage to the walls of blood vessels. Vascular wall damage provides a significant source of NGAL in serum through the activation of neutrophils [17].

Objectives

Taking into account the abovementioned reports, we aimed to investigate whether umbilical activin A and NGAL concentrations served as potential markers of perinatal hypoxia.

Material and methods

Subjects

A total of 58 newborns (gestational age: 37-41 weeks), admitted to the Department of Neonatology, Medical University of Silesia, Katowice, Poland between January 1, 2013 and August 30, 2013, were enrolled in the study. All neonates with congenital malformations, inborn errors of metabolism, blood group incompatibility, sepsis, diabetic mothers, and from multiple gestations were excluded from the study. Perinatal hypoxia was defined as the presence of at least two of the following conditions: intrapartum distress, indicated by fetal bradycardia with a heart rate of

Table I. Clinical characteristics of newborns.

Newborns (n= 58)		Hypoxic (n= 24)	Non-hypoxic (n= 34)	p#
Gestational age (wk)*		38 (37, 38)	38 (37, 38)	NS
Gender (%) female/male		50/50	48/52	NS
Apgar score** at 5 min.		5 (5, 6)	9 (8, 9)	<0.0001
Delivery* Vaginal delivery (%)		20	16.1	NS
Cesarean section (%)		80	83.9	NS
Blood gas analysis		24 (100%)	0	<0.001
pH*	≤ 7.2			
	> 7.2	0	34 (100%)	<0.001
HCO₃** (mmol/L)		15.6 (12.8, 19.2)	23.7 (22.6, 24.7)	<0.001
BE**		-11.4 (-14.7, -9.8)	-2.3 (-3.5, -1.3)	<0.0001
Lactate**		6.5 (5.8, 8.3)	1.8 (1.5, 2.2)	<0.001

* results shown as medians, (minimum and maximum values),
** results shown as medians and [confidence interval],
\# p-value from Mann-Whitney U test or Kruskal Wallis test.

Table II. Cord plasma levels (median, minimum, and maximum values) of activin A and NGAL in hypoxic and non-hypoxic babies.

	Hypoxic (n= 24)	Non-hypoxic (n= 34)	p#
Activine (ng/mL)	0.51 (0.32, 0.59)	0.27 (0.20, 0.48)	0.01
NGAL (ng/mL)	99.1 (68.7, 175.4)	22.3 (20.8, 25.1)	<0.001

*p value from Mann-Whitney U test.

<100 beats per min., late decelerations, or the absence of heart rate variability; Apgar score of ≤6 at 5 min.; the need for resuscitation for >1 min. with positive-pressure ventilation and oxygen immediately after birth; a pH value of ≤7.20 in the arterial cord blood; lactate concentration of ≥3 mmol/L; a base deficit of ≤ -12 mmol/L. Hypoxia was diagnosed in 24 out of the 58 newborns, while 34 babies, asymptomatic of perinatal hypoxia, constituted the control group.

Methods

Heparinized blood samples were obtained from the umbilical artery after cord clamping, immediately after delivery. Blood gas analysis was performed using Rapidlab 865 Blood Gas Analyzer (Siemens, Germany) immediately after blood sampling. All blood samples were centrifuged at 2000 rpm for 10 min. Serum was then stored at -80° C until assayed. Activin A concentrations were measured using specific two-site enzyme immunoassays (Serotec, Oxford, UK). The analytical detection limit of the activin A assay was <78 pg/mL; the intra- and inter-assay coefficients of variation were <10%. Cross-reactions for each assay with various inhibin-related proteins were approximately 1-5%. Activin A plates were read at 492 nm on an automated ELISA plate reader (Multiskan RC, Labsystems, Finland). NGAL concentrations were measured with the use of a sandwich enzyme immunoassay for the quantitative measurement of human lipocalin-2 (BioVendor).

Statistical analysis

Statistical analysis was performed using standard procedures available in STATISTICA10 (Statsoft Polska Inc.) and MedCalc Software Version 12.7.4. Normal distribution was tested using the Shapiro-Wilk test, while statistical significance differentiation between the two groups was assessed using the Mann- Whitney U test or Kruskal-Wallis tests. Quantitative variables are presented as median and confidence intervals, 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 diagnostic performance of serum NGAL and activin A was evaluated using receiver operating characteristic (ROC) curve analysis. The p-value of <0.05 was considered as statistically significant. The study was approved by the Local Ethics Committee and informed written parental consent was obtained before enrollment of each infant.

Results

Demographic and perinatal data of all newborns are shown in Table 1. No differences in gestational age, gender and mode of delivery between asphyxiated and non-asphyxiated babies were observed. However, newborns with symptoms of perinatal asphyxia had a significantly lower Apgar score at 5 min., lower pH, lower HCO_3, higher lactate concentration, and higher base deficit levels as compared to healthy neonates.

A significantly elevated activin A concentration was noted in the asphyxiated group as compared to controls (0.51 ng/mL [95% CI 0.32, 0.59] vs. 0.27 ng/mL [95% CI 0.20, 0.48]; p=0.01). Also, NGAL levels were increased in asphyxiated babies as compared to the control group (99.1 ng/mL [95% CI 68.7, 175.4] vs. 22.3 [95% CI 20.8, 25.1]; p<0.001) (Table II).

A negative correlation between NGAL concentration and Apgar score at 5 min., pH, HCO_3 based deficit, and lactate concentration was observed. Activin A was only negatively correlated with pH. There was a positive correlation between NGAL and activin A concentrations (R=0.54; p<0.01).

The ROC curve for NGAL had an area under the curve (AUC) of 0.922 [95% CI 0.85,0.97]; p<0.0001. The umbilical NGAL cutoff value of 33.9 ng/ml could differentiate asphyxiated from non-asphyxiated neonates, with a sensitivity of 100% and specificity of 78.3%, positive predictive value of 69% [95% CI 52.9,82.4] and negative predictive value of 100% [95% CI 92.3,100.0]. The ROC curve for activin A had an AUC of 0.584 [95% CI 0.47,0.68]; p=0.19. The umbilical activin A cutoff value of 0.208 ng/ml could differentiate asphyxiated neonates from non-asphyxiated neonates, with a sensitivity of 93.1% and specificity of 26.7%, positive predictive value of 38% [95% CI 26.7,50.4] and negative predictive value of 89% [95% CI 65.3-98.6].

Discussion

Over recent years, a number of investigators attempted to identify a biochemical marker able to identify perinatal asphyxia. The gold standard is to find a practical and sensitive marker to detect patients at risk and thereby take preventive or therapeutic measures in due time.

To date, none of the potential markers, including adrenomedullin (AM), S100 calcium binding protein B (S100B), neuronal specific enolase (NSE), glial fibrillary acid protein (GFAP), and vascular endothelial growth factor (VEGF), have been able to fulfil the necessary criteria. However, in the last decade, activin A has been in the limelight. A large body of evidence showing that brain lesions up-regulate the expression of activin A has been accumulated [8, 18, 19]. Furthermore, recent *in vivo* data have suggested that, in the presence of hypoxia and/or asphyxia, activin A concentrations increase in the central nervous system, similarly as in full-term infants who have suffered from asphyxia, as a result of developing brain damage, and were found to have increased activin A concentrations in the cerebrospinal fluid [20] and urine [21]. Our findings further confirmed this hypothesis, demonstrating that newborns with clinical signs of perinatal hypoxia had significantly higher levels of activin A and NGAL as compared to non-hypoxic infants. These results are comparable to our previous observations concerning activin A and NGAL in asphyxiated newborns [22, 23, 24]. Similar results were also demonstrated by other authors [10, 12, 19, 25]. Florio *et al.,* reported that hypoxic newborns had significantly higher activin A
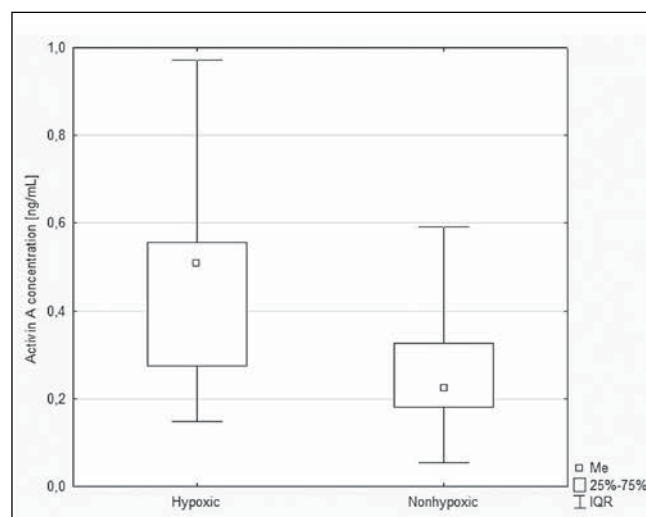

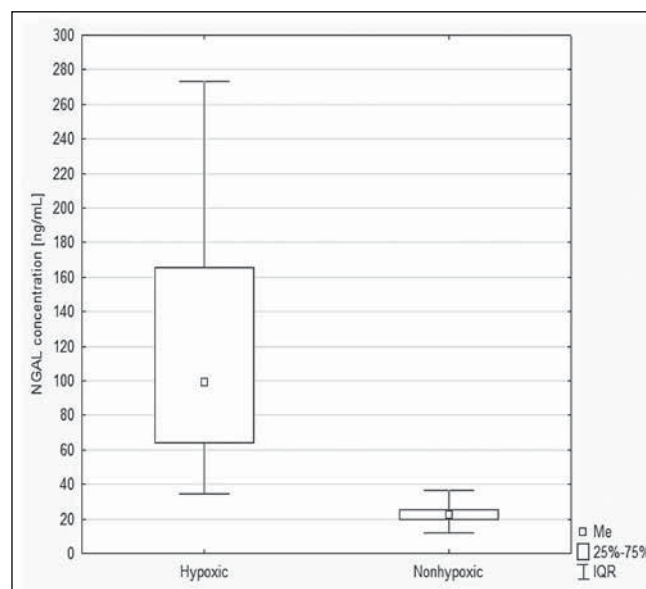**Figure 1.** Activin A concentration in hypoxic and non-hypoxic newborns*.

Figure 2. NGAL concentration in hypoxic and non-hypoxic newborns.

levels than non-hypoxic children, and that cord activin A levels were significantly correlated with other biochemical features of hypoxia, such as higher nucleated red blood cells and plasma hypoxanthine and xanthine levels, as well as lower pH and higher base deficit levels [19, 25]. However, other authors have been unable to show the usefulness of artery activin A concentration as a marker of feto-placental oxygenation and neonatal outcome. Tong *et al.,* suggested that umbilical artery activin A is not correlated with fetal pH, and only very little with fetal oxygenation [26]. On the basis of these observations, these authors suggested that the assumption concerning umbilical activin A concentration as a potentially useful biomarker indicating perinatal hypoxia and particularly hypoxic-ischemic encephalopathy in term infants would be highly unlikely. Noteworthy, it has been the largest study of fetal activin A levels reported until today.

In our study, we only observed an inverse relationship between activin A and umbilical vein pH, whereas higher concentration of NGAL in the asphyxiated group was correlated with lower pH, lower HCO_3, higher base deficit and higher lactate concentration, as well as lower Apgar score at 5 min., suggesting that NGAL could be an invaluable indicator of perinatal asphyxia. This suggestion is supported by other authors, claiming that asphyxiated neonates had significantly higher serum and urine NGAL concentrations as compared to controls [10]. Similarly, Raggal *et al.,* found that hypoxic newborns had significantly higher NGAL concentrations than non-hypoxic infants [12]. In their study, serum NGAL levels were also significantly higher in cases inclusive of acute kidney injury (AKI) than in those without AKI. Unfortunately, the design of our present study did not take AKI into account.

We found that NGAL showed 100% sensitivity and 78.3% specificity as a single marker of perinatal hypoxia in full-term newborns, contrary to activin A, whose sensitivity and specificity were 93.1% and only 26.7%, respectively. To the best of our knowledge, our study has been the first to demonstrate that NGAL is a better marker of perinatal hypoxia than activin A. In light of our observations, further investigation of NGAL in asphyxiated newborns, especially those with AKI, should be considered.

Conclusions

In conclusion, perinatal hypoxia is one of the most common factors responsible for increasing activin A and NGAL concentrations. Based on the high correlation of NGAL with clinical and biochemical signs of fetal and neonatal hypoxia, as well as higher sensitivity and specificity in differentiating between hypoxic and non-hypoxic babies, it seems safe to conclude that this protein could be a better marker of perinatal hypoxia than activin A.

Oświadczenie autorów:
1. Małgorzata Fiala – autor założeń pracy, zebranie materiału, przygotowanie manuskryptu i piśmiennictwa.
2. Małgorzata Baumert – autor koncepcji i założeń pracy, współautor tekstu pracy, korekta i akceptacja ostatecznego kształtu manuskryptu.
3. Piotr Surmiak – współautor tekstu pracy, zebranie materiału, analiza statystyczna wyników, współautor analizy i interpretacji wyników, przechowywanie dokumentacji – autor zgłaszający i odpowiedzialny za manuskrypt.
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5. Patrycja Sodowska – opracowanie wyników badań, korekta i aktualizacja literatury.

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