Clinical Biochemistry 74 (2019) 69–72

Contents lists available at ScienceDirect

Clinical Biochemistry

journal homepage: www.elsevier.com/locate/clinbiochem

Short Communication

Neuroapoptosis in newborns with respiratory acidosis at birth

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A B S T R A C T

Background: S100B protein is one of the most accurate biomarkers for diagnosis of neuroapoptosis and brain damage. The aim was to evaluate the lactate concentration and acid-base balance (pH, pCO_2, pO_2, HCO_3c and BEb) in umbilical cord blood to predict high risk of neuroapoptosis and analyze the relationship between the levels of these biomarkers and umbilical cord blood S100B protein concentration at birth.

Methods: Apparently healthy newborns were included. S100B protein and blood gas test (lactate and acid-base balance) were determined in umbilical cord blood at birth. Newborns were classified into two groups: with and without high risk of neuroapoptosis. Newborns with high umbilical cord blood S100B protein concentration were considered newborns at high risk of neuroapoptosis.

Results: Sixty-one newborns were included, 12 had high risk of neuroapoptosis and 49 did not. S100B protein concentration correlate directly with pCO_2 levels (Rho: 0.286, $p = .0321$) and lactate concentration (Rho: 0.278, $p = .0315$); and indirectly with pH (Rho: −0.332, $p = .01$). The analysis of the ROC curves yielded significant curves for pH and pCO_2 to predict high risk of neuroapoptosis, pH optimal cutoff value was 7.19 (sensitivity: 50%, specificity: 83.7%, AUC: 0.708); and pCO_2 optimal cutoff value was 60 mmHg (sensitivity: 30%, specificity: 85.4%, AUC: 0.705).

Conclusions: Respiratory acidosis is associated to high concentrations of S100B protein in umbilical cord blood at birth. Umbilical cord blood pH and pCO_2 may be useful in differentiating newborns at high risk of neuroapoptosis. Umbilical cord blood gas test may be valuable as risk indicator for neuroapoptosis at birth.

1. Introduction

Neuronal cell death can occur through neuronal cell apoptosis, or neuroapoptosis, which is the programmed death of neuronal cells. An increase in neuroapoptotic activity can cause brain damage. S100B protein is one of the most accurate biomarkers for diagnosis of neuroapoptosis and brain damage. In a healthy patient, serum S100B protein concentration is low and acts as a neurotrophic factor, however, levels in brain damaged patients increase and act as a neuroapoptotic factor [1–3]. High levels of serum S100B protein are associated with brain damage in adult patients with various diseases such as head trauma [4], cerebral ischemia [5] or cerebral hemorrhage [6]. Newborns with high levels of umbilical cord blood S100B protein have been associated with hypoxic-ischemic encephalopathy [7] or with indirect signs of asphyxia during birth [8].

Fetal hypoxia is related to neonatal morbidity and mortality. Reduced pH and increased lactate concentrations in umbilical cord blood are considered biomarkers of fetal hypoxia and predictors of neonatal morbidity and mortality [9].

The aim of this study was to evaluate the lactate concentration and acid-base balance [pH, partial pressure of carbon dioxide (pCO_2), partial pressure of oxygen (pO_2), bicarbonate concentrations (HCO_3c), base excess/deficit (BEb)] in umbilical cord blood to predict high risk of neuroapoptosis and analyze the relationship between the levels of these biomarkers and umbilical cord blood S100B protein at birth.

2. Materials and methods

2.1. Study design

This is a cross-sectional descriptive study carried out in Puerto Real University Hospital, Cádiz, Spain. The study was approved by the

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https://doi.org/10.1016/j.clinbiochem.2019.08.013
Received 30 April 2019; Received in revised form 11 August 2019; Accepted 28 August 2019
Available online 29 August 2019

Research Ethics Committee of Cadiz and adhered to the ethical recommendations of Declaration of Helsinki (Fortaleza, 2013). All participants signed an informed consent form.

2.2. Patients

Apparently healthy newborns were included between September and December of 2017. The following inclusion criteria were used: newborn from single pregnancy with gestational age at delivery between 37 and 41^{+6} weeks, birth weight between 10th and 90th percentile for gestational age and sex using the reference curve INTERGROWTH-21st and a 5-min Apgar score of 8 to 10. The exclusion criteria were: fetal malformations, congenital anomalies, gestational diabetes and hypertensive states of pregnancy (preeclampsia/eclampsia, chronic hypertension, chronic hypertension with superimposed preeclampsia, and gestational hypertension).

2.3. Sample analysis

Once delivery was completed, two arterial blood samples from the umbilical cord were taken: one sample in a pre-heparinized blood gas sampling syringe to determine lactate concentration and acid-base balance in the GEM Premier 4000 system by blood gas test (Werfen Group, Barcelona, Spain); and another sample in a tube with separator gel to obtain umbilical cord blood serum by centrifugation for 5 min at 4000 rpm and to determine S100B protein concentration in Hitachi Modular E170 autoanalyzer (Roche Diagnostics, Bassel, Switzerland). In addition, lactate concentration was determined by enzymatic method in Dimension EXL autoanalyzer (Siemens Healthcare Diagnostics, Marburg, Germany) to verify the result obtained by blood gas. A precision study (repeatability) of blood gas test and the method used for the determination of the S100B protein was performed by analyzing the same sample of umbilical cord blood 12 consecutive times.

2.4. Classification of patients

High levels of S100B protein are associated with increased neuroapoptotic activity and brain damage [1–8], but there are no studies indicating the reference ranges of umbilical cord blood S100B protein; however, there is a recent paper that provides the reference ranges of serum S100B protein in neurologically healthy newborns. The upper reference value was 0.51 μg/L for infants aged 0 to 4 months [10]. According to these reference values, newborns with S100B protein >0.51 μg/L could be associated with high risk of neuroapoptosis.

In this study, the newborns at high risk of neuroapoptosis were defined used only the concentration of umbilical cordon blood S100B protein. The newborns were classified into two groups: Newborns without risk of neuroapoptosis (newborns with S100B protein concentration ≤ 0.51 μg/L) and newborns at high risk of neuroapoptosis (newborns with S100B protein concentration > 0.51 μg/L).

2.5. Statistical analysis

Data were processed using MedCalc 13.0 (MedCalc Software, Ostend, Belgium), with significance set at $p < .05$. The methods used to measure of lactate were compared by Bland and Altman test, calculating the arithmetic mean of the differences between both methods. The precision study was performed by calculating the coefficient of variation (CV(%) = 100 x standard deviation / arithmetic mean). Quantitative variables were analyzed with the D'Agostino-Pearson test to determine whether they followed a normal (Gaussian) distribution or not. For the descriptive analysis, the frequencies of qualitative variables were used, as well as range and arithmetic mean for normally distributed quantitative variables and range and median for non-Gaussian quantitative variables. Correlations between normally distributed quantitative variables and between non-Gaussian variables were determined using the Pearson correlation coefficient and the Spearman rho correlation coefficient, respectively. Comparison of data sets was performed using the chi-squared test for qualitative dichotomous variables; Student's T distribution for quantitative variables with normal distribution, and the Mann-Whitney *U* test for non-gaussian distribution quantitative variables. We determined the accuracy of the independent variables to predict high risk of neuroapoptosis by analyzing receiver operating characteristic (ROC) curves, calculating area under the ROC curve (AUC), optimal cutoff value, sensibility and specificity. In order to reduce the number of false positives, optimal cutoff value was considered to be the one with the highest sensitivity and a specificity >80%.

3. Results

Sixty-one newborns were studied, 49 (80.3%) without risk of neuroapoptosis and 12 (19.7%) newborns at high risk of neuroapoptosis. All quantitative variables followed a non-gaussian distribution.

Table 1 shows demographic data and obstetric characteristics in both groups of newborns. Maternal age was significantly lower in newborns at high risk of neuroapoptosis ($p = .0103$). All newborns with high concentrations of umbilical cord blood S100B protein were male. No significant differences were obtained between both groups with maternal parity, gestational age, type of labor, application of oxytocin or epidural analgesia during labor or with weight or length of the newborn.

Table 1
Demographic data and obstetric characteristics.

	NBs without risk of neuroapoptosis ($n = 49$)	NBs at high risk of neuroapoptosis (n = 12)	*p* value
Maternal age [median(range)]	31.0 years (19–41)	27.5 years (17–36)	p = .0103*
Parity (n%)	Primiparous = 71% Multiparous = 29%	Primiparous = 83% Multiparous = 17%	$p > .05$**
Gestational age [median(range)]	39.2 weeks (37.0–41.4)	39.7 weeks (38.0–41.6)	$p > .05$*
Spontaneous delivery (n%)	31%	42%	p > .05**
Operative vaginal delivery (n%)	49%	33%	p > .05**
Cesarean section (n%)	20%	25%	p > .05**
Oxytocin administration (n%)	78%	92%	p > .05**
Epidural analgesia (n%)	88%	92%	p > .05**
NB gender (n%)	F = 41% M = 59%	F = 0% M = 100%	$p = .0185$**
NB weight [median(range)]	3350 g (1650–3930)	3195 g (2600–4330)	p > .05*
NB length [median(range)]	50.0 cm (38.5–55.0)	49.0 cm (47.0–52.0)	$p > .05$*

NB: newborn; F: females; M: males; *Mann-Whitney U test; **Chi-squared test.

Table 2
Descriptive statistical of umbilical cord blood biomarkers in newborns with and without high risk of neuroapoptosis.

	Group	Range	Median (CI 95%)	IQR	p value
S100B (μg/L)	0	0.157–0.510	0.278 (0.252–0.344)	0.147	
	1	0.623–1.750	0.906 (0.671–1.574)	0.595	
Lactate	0	1.8–9.6	4.8 (4.3–5.6)	1.9	p > .05*
(mmol/L)	1	2.4–13.1	5.3 (3.1–9.2)	4.5	
pH	0	7.15–7.55	7.28 (7.23–7.32)	0.13	*p* = .0061*
	1	7.16–7.29	7.20 (7.17–7.28)	0.10	
pCO_2	0	10–70	49 (46–54)	13	*p* = .0376*
(mmHg)	1	45–82	55 (49–67)	10	
HCO_3c	0	15.5–28.5	23.2 (21.9–24.7)	3.6	p > .05*
(mmol/L)	1	18.2–26.9	22.1 (18.9–26.7)	6.0	
BEb	0	−10.6-1.4	−3.5 (−5.5-(−3.0))	−4.8	p > .05*
(mmol/L)					
	1	−10.6-(−0.7)	−5.8 (−9.9-(−1.5))	−6.3	
pO_2	0	5–129	17 (14–21)	9	p > .05*
(mmHg)	1	7–34	13 (7–31)	13	

Group 0: Newborns without risk of neuroapoptosis; Group 1: Newborns at high risk of neuroapoptosis; CI: Confidence interval; IQR: Interquartile range; S100B: S100B protein; pCO_2: Partial pressure of carbon dioxide; HCO_3c: Bicarbonate; BEb: Base excess/deficit; pO_2: Partial pressure of oxygen; *Mann-Whitney U test.

In the precision study, the CVs of blood gas test were 0.1%, 3.1%, 2.9% and 3.2% for pH, pCO_2, pO_2 and lactate respectively; and 1.1% for the umbilical cord blood S100B protein concentration. Umbilical cord blood lactate concentrations obtained by blood gas test and enzymatic method were similar; the arithmetic mean of the differences was 0.19 mmol/L (4.4%). The differences between both methods were statistically insignificant.

Descriptive statistical of umbilical cord blood biomarkers in both newborns groups can be compared in Table 2. The only significant differences between the two groups of newborns were obtained with pH and pCO_2.

S100B protein concentration correlated directly with pCO_2 levels (Rho: 0.286, $p = .0321$) and lactate concentration (Rho: 0.278, $p = .0315$); and indirectly with pH (Rho: −0.332, $p = .01$). No significant correlation was obtained between S100B protein and the rest of biomarkers.

The analysis of the ROC curves yielded significant curves with umbilical cord blood pH and pCO_2 to predict high risk of neuroapoptosis. AUCs were 0.708 and 0.705 for pH and pCO_2 respectively. The optimal cutoff value of pH was 7.19 (sensitivity: 50% and specificity: 83.7%) and pCO_2 optimal cutoff value was 60 mmHg (sensitivity: 30% and specificity: 85.4%). No significant differences resulted between AUCs obtained with both biomarkers ($p > .05$).

4. Discussion

This study shows that S100B protein levels in newborns with respiratory acidosis are higher than in newborns with normal acid-base balance. This suggests that these newborns, even in the absence of clinical signs of perinatal hypoxia, exhibit some degree of neuroapoptotic activity.

Multiple studies have related umbilical cord arterial acidosis with neonatal morbidity and mortality, hypoxic-ischemic encephalopathy, intraventricular hemorrhage and cerebral palsy [11]. Some authors associate more frequently neonatal complications to metabolic acidosis rather than to respiratory acidosis, as they consider that in the first phases of fetal hypoxia, respiratory acidosis takes place (pCO_2 increases and BEb remains within the reference values), followed by metabolic acidosis when fetal hypoxia is prolonged, increasing the products of anaerobic metabolism such as lactate [9,11,12].

This study shows that pH and pCO_2 levels can be useful to differentiate newborn with an increased risk of neuroapoptosis, even in the absence of clinical signs. The ROC curve analysis showed the usefulness of pH and pCO_2 to detect newborns with high levels of S100B protein in umbilical cord blood. The determination of pH ≤ 7.19 or pCO_2 > 60 mmHg in umbilical cord blood was a very specific value to differentiate newborn at high risk of neuroapoptosis but with low sensitivity.

These results manifest an association between neuroapoptosis and respiratory acidosis in newborn at birth. The acidosis produced by hypercapnia can be a physiopathological mechanism that causes neuronal death, implying a possible neurotoxic effect of carbon dioxide. This effect was tested in animal brains by injecting them carbon dioxide [13].

Various papers have shown that lactate concentration in umbilical cord blood can be a useful biomarker to predict morbidity in neonates [14]. In this study there was a significant correlation of the S100B protein with lactate in umbilical cord blood, however this biomarker did not yield a significant AUC to differentiate between newborns with and without high risk of neuroapoptosis, probably because the newborns at high risk of neuroapoptosis of this study did not suffer a prolonged fetal hypoxia so as to originate a significant increase in lactate concentration. As for BEb, some authors relate the base deficit in umbilical cord blood (>12 mmol/L) with a higher risk of neonatal complications [15]. In this study there was no relationship between S100B protein and BEb.

The pH and pCO_2 in umbilical cord blood are biomarkers that can be determined at the point of care by simple and rapid procedures as a blood gas test. The results obtained in this study reinforce the importance of the umbilical cord blood gas test at birth, as not only do they act as biomarkers of fetal hypoxia and predictors of neonatal morbidity and mortality, they also may be used as risk indicators for neuroapoptosis and potential brain damage. Although the newborns at high risk of neuroapoptosis had no clinical manifestations of serious sequels, it is necessary to differentiate newborn with possible brain damage in order to reexamine and accomplish further evaluations as required.

One limitation of this study was to use the upper reference value of serum S100B protein (0.51 μg/L) to define newborns at high risk of neuroapoptosis. There are no published papers indicating the cutoff value of umbilical cord blood S100B protein to predict neuroapoptosis, but a large number of studies have associated high levels of S100B protein with a high risk of neuroapoptosis [1–8]. All newborns with high S100B protein concentration were male, this could suggest the need for gender-specific S100B protein reference intervals, although it may be due to the small number of newborns at high risk of neuroapoptosis ($n = 12$). This study is considered preliminary and additional studies are needed to confirm the results obtained.

In relation to the precision study, all the CVs of blood gas test and the concentration of S100B protein in umbilical cord blood complied with the manufacturer's specifications for peripheral blood (CV for S100B protein <1.4% and CV values below 2.0%, 7.1%, 4.6% and 10% for pH, pCO_2, pO_2 and lactate respectively).

In conclusion, respiratory acidosis is associated to high concentrations of S100B protein in umbilical cord blood at birth. Umbilical cord blood pH and pCO_2 may be useful in differentiating newborns at high risk of neuroapoptosis and possible brain damage. Umbilical cord blood gas test may be valuable as risk indicator for neuroapoptosis at birth.

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