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Urinary S100A1B and S100BB to predict hypoxic ischemic encephalopathy at term

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1. ABSTRACT

Urinary S100A1B and S100BB were measured to detect cases at risk of hypoxic-ischemic encephalopathy (HIE) in asphyxiated newborns. We recruited 42 asphyxiated infants and 63 healthy term neonates. S100A1B and S100BB were measured at first urination (time 0) and at 4 (time 1), 8 (time 2), 12 (time 3), 16 (time 4), 20 (time 5), 24 (time 6), 72 (time 7) hours after birth. 20 infants had no/mild HIE with good prognosis (Group A) and 22 had moderate/severe HIE with a greater risk of neurological handicap (Group B). Urine S100A1B and S100BB levels were significantly (P less than 0.0.01, for all) higher at all monitoring time-points in Group B than Group A and controls, but not between Group A and controls. Both S100A1B and S100BB have great sensitivity and specificity for HIE since their first measurement. In conclusion, S100A1B and S100BB are increased in urine collected from asphyxiated newborns who will develop HIE since first urination, and their measurement may be useful to early predict HIE when monitoring procedures are still of no avail.

2. INTRODUCTION

Protein S100 is a family of dimeric acidic calcium-binding protein of the EF-hand family constituting a major component of the cytosol of various cell types (1), of whom proteins S100A1B and S100BB collectively denoted S100B when detected using S100B specific antibodies) are predominantly concentrated in the central nervous system (CNS). Here they are mainly located in glial cells, in Schwann cells (2, 3), but also in specific neurons sub-populations as well as in neural precursor cells (4-6). Although the biological role of S100B has not been completely clarified, it has been reported to regulate several cellular functions (cell-cell communication, cell growth, cell structure, energy metabolism, contraction and intracellular signal transduction) at physiological concentrations, while it has been shown to be neurotoxic at high concentrations (7). Elevated S100B concentrations in biological fluids such as cerebrospinal fluid, cord blood, amniotic fluid and, peripheral blood and urine have also been shown to represent a marker of brain damage (8-14). In this respect, since S100B is mainly eliminated by the

kidneys (15), data relating to the urine of newborns developing intraventricular hemorrhage and adverse neurological outcome support the expedience of the clinical use of repeated S100B measurements in these patients (11-14).

In the present study we investigated whether measurements of urine levels of S100 dimers (S100A1B and S100BB) in asphyxiated full-term newborns may constitute a useful tool for the early detection of cases at risk of Hypoxic-ischemic encephalopathy (HIE). Indeed, HIE constitutes an important cause of neonatal mortality and of permanent neurological disabilities in full-term newborns (16, 17). Despite technological improvement, in perinatal monitoring, the incidence of HIE has remained constant over the past four decades (1-6 per 1000 live births) as well as the occurrence of neurological handicap. The explanation of such evidence may reside in the fact that: i) the time-window for therapeutic intervention, to reduce delayed neuronal death or programmed cell death after birth asphyxia, is restricted within the first 6-12 hours; ii) perinatal standard monitoring procedures (i.e. fetal heart monitoring, umbilical artery acidemia, Apgar scores) have been reported to be of limited value (18); iii) imaging techniques (i.e. cranial ultrasound and MRI) are useful for prognosis, but not until 24 hours or more after birth and continuous EEG recordings can be of value within the first 24 hours (19); iv) neonatal intensive care (NICU) treatment (i.e. mechanical ventilation, sedation) may affect monitoring parameter results. Taken together, the possibility to measure brain-specific biochemical markers such as S100A1B and S100BB able to offer additional support in the early detection of cases at risk of HIE, at stages when monitoring procedures are still of no avail could be especially useful.

3. MATERIALS AND METHODS

3.1. Subjects

The study was performed at tertiary referral Centres for obstetrics and neonatal intensive care units between October 2003 and January 2007. We recruited approximately 40 infants in the asphyxiated group (full-term asphyxiated infants with a gestational age more than 36 weeks) and 63 healthy infants as control. This decision was based on the number of deliveries at our Centres and epidemiological studies relating to the incidence of asphyxia in our countries, so that we expected approximately one third of asphyxiated infants to exhibit moderate or severe HIE. Informed consent was obtained from all parents of the patients prior to inclusion in the study. Approval was obtained from the Human Investigations Committees of the participating institutions.

Therefore, in the study we included:

a) forty-two consecutive infants with perinatal asphyxia who were born in our hospitals. All asphyxiated newborns were delivered by emergency cesarean section due to acute fetal distress, defined according to the American College of Obstetricians and Gynecologists as non-reassuring fetal status (bradycardia, late deceleration of the fetal heart rate, severe and repetitive variable deceleration of the fetal heart rate, reduced beat-to-beat variability) (20). Asphyxia was defined according to an Apgar score less than 3 at the 5th minute, pH less than 7.0., or BE less than -12 in cord blood or venous blood taken from newborns within 60 min of birth, or the need for positive pressure ventilation (more than 3 minutes) (20). Infants that fulfilled 3 or more of the above clinical and biochemical criteria were included in the asphyxia group. The asphyxia group was retrospectively subdivided according to the clinical examination: no or mild HIE with good prognosis, and moderate or severe HIE with a greater risk of neurological handicap (21);

b) sixty-three healthy term neonates at the same gestational age delivered consecutively to the cases both by elective cesarean section (n= 26) or vaginally (n= 37) represented the controls. In these infants clinical and laboratory parameters were recorded at birth and at 24 hours from birth for the standard assessment (i.e. RBC count, glycaemia, urea, creatinine and ions concentration). In 37 out 63 healthy infants developing jaundice it was possible to investigate in the post-natal period (i.e. 4, 8, 12 hours) bilirubin blood levels and blood ph (including ions concentrations).

Exclusion criteria from the study were: any malformative syndrome, systemic infections, intrauterine growth retardation, or cardiac or hemolytic disease. Other exclusion criteria were: multiple pregnancies, congenital or perinatal infections, maternal drug addiction, maternal hypertension and diabetes.

In all of the asphyxiated newborns cerebral ultrasound scanning was recorded and neurological examination assessed at the time of urine collection by a single examiner in each Center, who did not know the results of the urine test. Blood was drawn by means of a catheter inserted in the cubital vein at birth in order to monitor clinical and laboratory parameters.

3.2. Cranial Assessment

Standard cerebral ultrasonography was performed by a real-time ultrasound machine (Acuson 128SP5, Mountain View CA, USA) using a transducer frequency emission of 3.5. MHz. In the controls cerebral ultrasound patterns were evaluated before discharge from the hospital 72 hours from birth.

3.3. Neurodevelopmental outcome

In the asphyxiated group, the presence within the first 7 days after birth of HIE was classified according to the criteria described by Sarnat and Sarnat (22). HIE was defined as mild if hyperexcitability or hypotonia persisted without seizures for at least 72 hours after birth; as moderate if the infant was lethargic and had hypotonia, weak primitive reflexes, and seizures; and as severe if the infant showed frequent seizures, apnea, flaccid weakness, or coma. EEG traces were recorded in the asphyxiated infants within 7 days from birth.

Table 1. Perinatal data in the preterm and term groups. Data are given as means +/- SD.

Perinatal clinical characteristics	Asphyxia Group (n= 42)	Control Group (n=63)
Maternal Age (y)	29.2 +/-3.1	28.4 +/- 4.4
Caesarean Section (n°)	42	26
Gestational Age more than 36 wks (n°)	42	63
Birthweight (g)	3,111 +/- 314	3,341 +/- 211
Male/Female (n°)	19/17	32/31
Factors associated with primary outcomes		
Apgar score less than 3 at 1st min	36	0
Apgar score at less than 3 at 5th min	36	0
Respiratory Distress Syndrome N/total	11/42	0/63
Mechanical Ventilation N/total	36/42	0/63
Inotrope Therapy N/total	39/42	0/63
Neurological Examination at Admission		
• Normal	0	0
• Suspect	24	0
• Abnormal	0	0
Cerebral Ultrasound Pattern		
• IVH grade 2	1	0
• IVH grade 3	2	0
• IVH grade 4	0	0
Death within the 7th day from birth	3	0

Neurological examination was performed at the same time-points as urine sampling. Neonatal neurological conditions were classified using a qualitative approach as described by Prechtl (23), assigning each infant to one of three diagnostic groups: normal, suspect or abnormal. An infant was considered to be abnormal when one or more of the following neurological syndromes were present: hyper- or hypokinesia, hyper- or hypotonia, hemisyndrome, apathy syndrome, hyperexcitability syndrome. An infant was classified as suspect if only isolated symptoms were present but no defined syndrome.

3.4. S100A1B and S100BB urine measurement

S100 dimers level in urine was measured at first urination (time 0), and 4 hours (time 1), 8 hours (time 2), 12 hours (time 3), 16 hours (time 4), 20 hours (time 5), 24 hours (time 6), and 72 hours after birth (time 7). In the asphyxiated infants a catheter was inserted into the bladder for urine sampling, owing to their critical clinical conditions and to the effects of sedative drugs. Control infants were monitored by S100 dimers measurement in the urine at the same time-points as the asphyxiated group, using a standard urine collector. In the control group were included only those infants in whom spontaneous urination occurred at least 4 times during the collection time: time 0 (n= 59), time 1 (n= 56), time 2 (n= 54), time 3 (n= 55), time 4 (n= 58), time 5 (n= 51), time 6 (n= 56), time 7 (n= 56).

At the indicated time-points urine samples were immediately centrifuged at 900g for 10 min, and the supernatants stored at –20°C before measurement. The S100B protein concentration was measured in all samples, using dimer-specific, commercially available immunoassays (CanAg S100A1B EIA and CanAg S100BB EIA Fujirebio Diagnostics AB, Gothenburg, Sweden). The assays allowed to determine S100A1B and S100BB concentrations in urine without cross-reactivity with other forms of S100. Calibration curves are constructed for each assay by plotting absorbance value versus the concentration for each calibrator. Each measurement was performed in duplicate according to the manufacturer's recommendations and the averages were reported. The sensitivity of the S100A1B assay was 1.1.5 µg/L, and the coefficient of variability was 5.2. % or lower within-assay and 8.0.% or lower inter-assay. The sensitivity of the S100BB assay (B_0 +/- 3SD) was 1.2. µg/L, and the coefficient of variability was 4.5. % or lower within-assay and 7.5.% or lower inter-assay.

3.5. Statistical Analysis

Data on neonatal outcomes and laboratory parameters were analyzed by Turkey one-way ANOVA and Mann-Whitney U test when not normally distributed. Comparison between proportions was performed using Fisher's exact test. S100A1B and S100BB values were expressed as mean +/- SE and differences at the various time-points were evaluated by the Mann-Whitney U test when two groups were compared, and by the Kruskal-Wallis test for repeated measurements.

The sensitivity, specificity, predictive value and likelihood ratios of S100A1B and S100BB as diagnostic tests for the detection of HIE in asphyxiated newborns were assessed by using the Receiver Operating Curve (ROC) test (24). Statistical analysis was performed using the GraphPad Prism version 3.0.0 for Windows (GraphPad Software, Inc., San Diego, California, USA), and statistical significance was set at P less than 0.0.5.

4. RESULTS

Perinatal characteristics in the studied groups are shown in Table 1. No statistical significant differences (P more than 0.0.5, for all), between groups, have been found for maternal age, age and weight at birth and gender. Of the 42 asphyxiated infants 20 had no or mild HIE with good prognosis (Group A), and 22 had moderate or severe HIE with a greater risk of neurological handicap (Group B).

Table 2 shows the clinical and biochemical characteristics recorded at birth in the three groups studied. As expected, there were significant differences (P less than 0.0.01, for all), between groups, regarding the incidence of emergency caesarean section, Apgar score at 1' and 5' minute, the need of primary resuscitation and of inotrope

Table 2. Laboratory parameters recorded at admission in the apshyxiated and control groups. Data are expressed as median +/- SD

	Asphyxia Group (n= 42)	Control Group (n=63)
Red blood cell count (10^6/mm^3)	3.94 +/- 0.6	3.95 +/- 0.5
Hemoglobin (g/dL)	13.7 +/- 0.3	13.6 +/- 0.4
Hematocrit rate %	41.2 +/- 2.4	40.5 +/- 2.2
Venous blood pH	7.00 +/- 0.1[1]	7.35 +/- 0.2
Partial venous CO_2 pressure (mmHg)	66.3 +/- 15.5	43.7 +/- 3.9[1]
Partial venous O_2 pressure (mmHg)	37.1 +/- 4.6	39.6 +/- 3.4
Base excess	-14.3 +/-3.3[1]	0.2 +/- 2.1
Na^+ (mmol/L)	138 +/- 5	140 +/- 3
K^+ (mmol/L)	4.4 +/- 0.2	4.3 +/- 0.2
Ca^{++} (mmol/L)	1.12 +/- 0.08	1.12 +/- 0.1
Plasma glucose (mmol/L)	4.2 +/- 1.3	4.1 +/- 1.4
Urea	39.2 +/- 11.8	40.6 +/- 9.6
Creatinine	0.89 +/- 0.23	0.91 +/- 0.19
Urine gravity	1,014 +/- 2.4	1,012 +/- 3.3

[1]P less than 0.05

therapy, the incidence of acute respiratory distress syndrome and the occurrence of mechanical ventilation support. Laboratory results showed that venous blood pH, mixed venous carbon dioxide tension and base excess were significantly different (P less than 0.0.1, for all) in asphyxiated newborns compared with control infants regardless of the severity of HIE. No differences (P more than 0.0.5, for all) were found between the two asphyxiated subgroups in the incidence of acute respiratory distress syndrome (group A, 6/22; group B, 5/20), clinical and laboratory monitoring parameters, or in cerebral ultrasound scans. There were significant differences (P less than 0.0.1, for all) in blood pH, mixed venous carbon dioxide tension, and base excess between the asphyxiated groups and control infants 4 and 8 hrs after birth, whereas urine gravity did not statistically differ (P more than 0.0.5).

At 12 hours from birth there were no differences in laboratory findings, except for base excess (Group A: -1.4. +/- 0.7.; Group B: -6.1. +/- 1.7.; Controls: 1.4. +/- 0.2.; P less than 0.0.5). No differences were found in laboratory monitoring parameters between the asphyxiated infants at different monitoring time-points (i.e. 20, 24 and 72 hours time-points) and between the asphyxiated infants and controls (i.e. 24 hours time-point), except for cerebral ultrasound patterns. At 12 hours cerebral ultrasound was negative for cerebral bleeding in all but 6 Group B infants (intraventricular hemorrhage n= 1; intraventricular hemorrhage with ventricular dilatation n= 2), who later died. Periventricular hyperechogenicity was observed in 34 out of 42 asphyxiated infants (Group A: n= 14; Group B: n= 20; p less than 0.0.5). Identical cerebral echographic patterns were observed at 72 hours. In the controls cerebral ultrasound patterns were negative for bleeding or other central nervous system diseases.

Twenty-four of 42 asphyxiated infants were classified as suspect at neurological examination on admission and at different monitoring time-points (Group A: n= 5 hypo-hypertonia, n= 5 hyperexcitability; Group B: n= 7 hyperexcitability, n= 7 hypo-hypertonia; ns).

4.1. Urine S100A1B and S100BB levels and, the prediction of HIE

Both S100A1B and S100BB were measurable in all samples evaluated. The mean hour collection for first void was 2 hours (range: 1-3 hours). In details, S100A1B levels do not change throughout the monitoring time-points in urine samples collected both from healthy controls and newborns affected by perinatal asphyxia who had no or mild HIE with good prognosis (Group A). On the contrary, levels in Group B changed at each time point evaluated, since they were highest and lowest at time-point 1 (4 hrs from birth) and 7 (72 hrs from birth), respectively (P less than 0.0.001, for both) (Figure 1). When evaluated according the presence of HIE, levels in Group B were significantly (P less than 0.0.01, for all) higher at all monitoring time-points than Group A HIE infants and controls, except at 72 hours from birth (time 7) when were superimposable (P more than 0.0.5, for all) in the three studied groups.

With respect to S100BB, levels were unchanged throughout the monitoring time-points in healthy (controls) and Group A newborns, however in Group B lowest (P less than 0.0.01) concentrations were detected at 72 hours from birth (time 7) (Figure 1). Moreover, S100BB urine levels in Group B HIE infants were significantly higher (P less than 0.0.01, for all) at all monitoring time-points than Group A HIE infants and controls except at 72 hours (time 7) when were superimposable (P more than 0.0.5, for all) in the three studied groups (Figure 1).

Of the 42 asphyxiated infants, 22 had moderate or severe HIE (Group B), making an overall prevalence of the disease in our population of 20.9.5% ($CI_{95\%}$: 11.6.6-30.2.4%). This was the predicted probability of developing brain damage before S100A1B and S100BB measurements were performed (pre-test probability).

Table 3 shows the predictive values of S100A1B and S100BB protein levels at different monitoring time-points for the occurrence of HIE in asphyxiated infants. By using the ROC curve analysis, the specificity, sensitivity, positive (PPV) and negative predictive (NPV) values, positive and negative likelihood ratio and, area under the ROC curve of serial urinary S100A1B and S100BB levels as diagnostic tests for early prediction of HIE in asphyxiated newborns were computed (Table 3). As can be seen, both S100A1B and S100BB have great sensitivity and specificity since their first measurement (i.e. at the first urination) (Table 3).

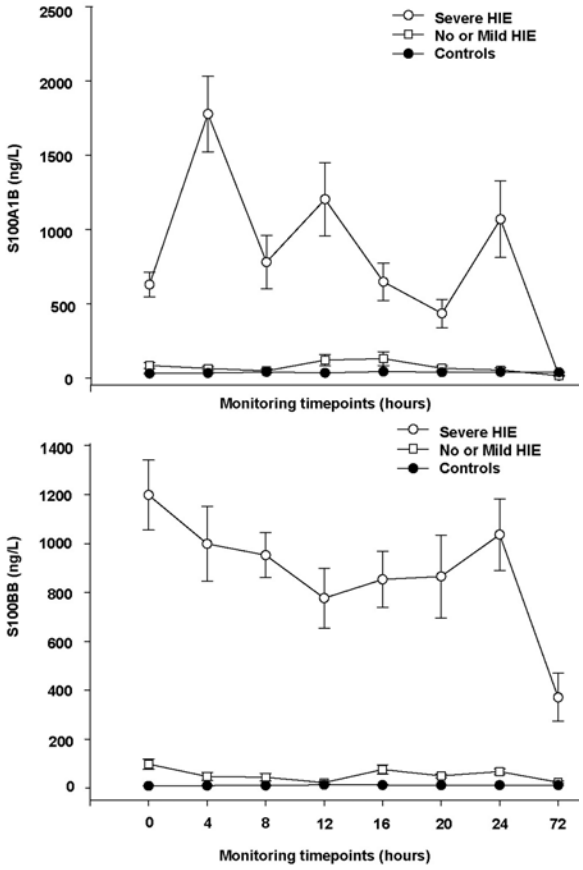

Figure 1. S100A1 and S100BB urine concentrations (ng/L) in asphyxiated infants sub-grouped in no/mild HIE and moderate/severe HIE groups and controls throughout the monitoring time-points.

5. DISCUSSION

The present study shows that urine S100 concentrations in the two dimers assessed at the different monitoring time-points were higher in the full-term asphyxiated infants who developed severe HIE than in those who did not at follow-up or in controls. The finding is not surprising since A1B dimer has been previously shown to be located in addition to nervous tissue, in other extra-nervous cell-types (1-4). For example, this holds for white fat, skeletal muscle (slow-twitch skeletal muscle fibers), heart (cardiomyocytes), liver, spleen and kidney (renal tubule cells), that constitutes the majority of the S100 A1B concentrations found in human tissues (4,5). Therefore, it is reasonable to suppose that part of the amount of S100A1B concentrations detected in urine, at this stage, can derive from the several organs including the central nervous system. Although the relevance of A1B concentration could be limited as a brain damage marker, especially in the mild and moderate HIE, in severe HIE A1B can exert a key-role as a early warning signal for the occurrence of multi-organ failure that constitutes the main dramatic complication in the post-asphyxia period. This especially holds for transient myocardial insufficiency and for oligo-anuria that can constitute a common repertoire in the early phases after an hypoxic-ischemic insult.

The elevated concentrations of BB dimer in urine are consistent with previous reports on sick infants such as preterm newborns complicated by cerebral bleeding and by acute and chronic hypoxia (25). However, elevated BB dimer concentrations in severe HIE infants warrant further consideration. Indeed, the fact that i) BB dimer is brain specific, together with the evidences that ii) the kidneys do not produce BB and that iii) due to its low molecular weight BB cannot be urine gravity dependent; together support the hypothesis that the reason by which BB is detected in high concentrations in urine reasonably refers to an exaggerated release of the protein from damaged CNS into systemic circulation. This hypothesis is further reinforced by data reporting that BB can be released from CNS under different conditions related with a patho-physiological cascade of events that can lead to brain damage (26-28). Of note, both in human and in animal models an early increase of the protein (within 15' minutes from insult) has been shown during hypoxia stress associated with cerebrovascular hemodynamic changes and brain damage (26-29). Same findings have been reported under chronic hypoxia condition, such as fetal growth restriction, suggesting that the protein can be regarded as a early marker of fetal/neonatal hypoxia. Changes in brain blood barrier permeability after acute-chronic hypoxia insults have been related to adaptive mechanisms activated by cerebrovascular autoregulation system.

The mechanism trough which the protein can be involved in the cascade of events triggering or not perinatal brain damage is biphasic: S100 at nanomolar concentrations stimulates neurite outgrowth and enhances survival of neurons during development (29), whilst micromolar levels of extracellular S100 *in vitro* stimulate the expression of proinflammatory cytokines and induce apoptosis. On the other hand, cell-based and clinical studies have implicated S100 in the initiation and maintenance of a pathological, glial-mediated proinflammatory state in the CNS. Over-expression of S100B increases vulnerability to cerebral hypoxic-ischemic injury, as S100B transgenic mice subjected to hypoxia-ischemia showed a significant increase in mortality, more extensive cerebral injury and neuroinflammation in response to injury. Therefore, the possibility that some of the S100BB derives from this process and participates in the pathological cascade of events responsible for brain damage hypoxia-mediated has to be taken into account.

Data from the present study would support S100A1B and S100BB measurement in sick infants in the perinatal period as an additional tool for early detection of cases at high risk of brain damage when standard monitoring procedures can be of no avail. Indeed, on this regard S100A1B and S100BB had great sensitivity and specificity since their first measurement (i.e. at the first urination) (Table 3). The finding could be especially useful bearing in mind that the time-window for therapeutic strategies in full term asphyxiated infants is restricted to the first 6-12 hours after birth.

Table 3. S100A1B and BB concentrations (ng/L) in urine of asphyxiated infants sub-grouped in no or Mild HIE and moderate/severe HIE groups and in controls at different time-points

S100A1B Urine Concentration (ng/L)									
	Moderate/Severe HIE			**No or Mild HIE**			**Controls**		
Monitoring Timepoint	Median	5°	95°	Median	5	95°	Median	5°	95°
First urination	555.0	455.0878	803.618	30.8	42.86451	125.6829	30.95	26.92352	33.0281
4 hours (1)	2854.40	1495.236	2600.006	17.2	32.56758	95.32506	30.20	31.42835	34.80005
8 hours (2)	446.05	408.31	1152.708	32.9	20.87781	74.9264	42.875	39.11806	41.23163
12 hours (3)	888.0	656.7532	1749.756	26.9	44.23	198.1132	29.70	32.90343	36.73463
16 hours (4)	999.0	900.5158	1623.122	26.4	31.42096	229.8601	43.30	42.88256	43.40744
20 hours (5)	555.0	565.7674	1276.644	38.4	29.99323	101.5331	37.30	37.57518	39.75999
24 hours (6)	666.0	507.2557	1630.837	30.5	23.05599	84.15348	42.90	39.78687	41.60475
72 hours (7)	333.0	184.2094	875.5553	13.3	10.08397	21.78656	43.30	36.18880	39.99090
S100BB Urine Concentration (ng/L)									
Monitoring Timepoint	Median	5°	95°	Median	5°	95°	Median	5°	95°
First urination	1114.90	897.3451	1501.511	151.30	53.95156	141.5874	8.115	8.110629	8.723563
4 hours (1)	832.50	736.9168	1386.458	13.60	11.25959	82.70041	10.40	9.350560	10.2246
8 hours (2)	858.55	761.946	1143.909	23.0	10.19175	77.3672	9.35	9.441156	10.53981
12 hours (3)	733.35	517.2234	1036.765	10.0	12.19082	31.49023	13.85	11.7092	13.65563
16 hours (4)	675.50	646.342	1128.228	58.70	34.44188	116.3486	11.38	10.86494	12.59054
20 hours (5)	666.0	554.8575	1241.428	50.70	34.48462	64.58065	10.45	10.37935	12.09904
24 hours (6)	888.0	731.4808	1342.328	63.70	43.14229	90.74403	10.35	10.49189	12.31166
72 hours (7)	285.60	342.6686	999.0678	14.40	14.59289	32.11238	10.20	10.45521	12.27899

Values are expressed as median and 5°-95° centiles. Urinary S100A1B and S100BB to predict hypoxic ischemic encephalopathy at term

Table 4. Sensitivity, specificity and predictive values of serial urinary S100A1B and S100BB levels as diagnostic test for early prediction of HIE in asphyxiated newborns

Monitoring Time-point	**S100A1B (ng/mL) Cut-off value**	**Sens (%) (95% C.I.)**	**Spec (%) (95% C.I.)**	**PPV (%)**	**NPV (%)**	**LR (+)**	**LR (-)**	**AUC**
First urination	186.6	100 (80,3-100,0)	96.3 (89,5-99,2)	85	100	27.00	0.00	0.998
4 hours (1)	131.9	100 (80.3-100)	96.4 (89.5-99.3)	86	88.9	37.23	0.05	0.997
8 hours (2)	112.9	100 (84.4-100)	97.5 (91.3-99.6)	91.7	100	40.5	0.00	0.998
12 hours (3)	333	100 (71.3-100)	97.3 (90.6-99.6)	84.6	100	37.0	0.00	0.998
16 hours (4)	44.4	65.7 (47.8-80.9)	91.4 (83.0-96.4)	76.7	86.0	7.6	0.38	0.683
20 hours (5)	44.5	68.6 (50.7-83.1)	90.1 (81.5-95.6)	75.0	86.9	6.9	0.35	0.712
24 hours (6)	60.4	67.7 (48.6-83.3)	94.1 (86.8-98.0)	80.8	88.9	11.5	0.34	0.746
72 hours (7)	44.6	45.16 (27.3-64.0)	98.82 (93.6-99.8)	93.3	83.2	38.4	0.55	0.536
Monitoring Time-point	**S100A1B (ng/mL) Cut-off value**	**Sens (%) (95% C.I.)**	**Spec (%) (95% C.I.)**	**PPV (%)**	**NPV (%)**	**LR (+)**	**LR (-)**	**AUC**
First urination	9.69	100 (65,1-96,9)	97.8 (90,9-100,0)	100	92.9	33.68	0.14	0.896
4 hours (1)	10.96	95.5 (77.1-99.2)	90.9 (90.9-100)	100	88.9	37.23	0.05	0.979
8 hours (2)	12.6	86.4 (65.1-96.9)	100 (90.9-100)	100	92.9	33.68	0.14	0.867
12 hours (3)	18.4	86.2 (68.3-96)	100 (90.7-100)	100	90.5	32.76	0.14	0.876
16 hours (4)	18.4	90.0 (73.4-97.8)	100 (90.7-100)	100	92.7	34.2	0.1	0.942
20 hours (5)	18.4	96.7 (82.7-99.4)	97.4 (86.5-99.6)	96.7	97.4	37.7	0.03	0.974
24 hours (6)	18.4	96.7 (82.7-99.4)	100 (90.9-100)	100	97.5	18.85	0.03	0.977
72 hours (7)	18.4	80 (61.4-92.2)	100 (90.9-199)	100	86.7	31.2	0.2	0.862

PPV: positive predictive value; NPV: negative predictive value; Sens: sensitivity; Spec: specificity; LR: positive (+) and negative (-) likelihood ratio; AUC: area under the ROC curve

S100A1B and S100BB belong to the protein S100-family of dimeric acidic calcium-binding proteins, both collectively denoted S100B when detected using S100B specific antibodies (1). Therefore, the main differences between the present and previous series related to S100B measurement regarded protein assessment technique: i) a different S100 assessment based on immunoluminometric vs. ELISA method; ii) an assay specific for the B subunit of the S100 protein defined by three monoclonal antibodies SMST 12, SMSK 25 and SMSK 28 instead of an assay BB dimer specific defined by three monoclonal antibodies S10 or catcher antibody (combined with S52 or detecting antibody), S12 and S13; iii) the assessment in different biological fluids such as urine of which the present series constitute the first observation in sick infants. Taken together, the present findings offer additional support to the use of S100 protein both B and/or BB and A1B dimers in sick infants at risk of perinatal brain damage independently from the assessment methods. The choice for using one method despite the other, that is not matter of the present investigation, is related with infrastructure facilities, reproducibility, costs and time session for measurement.

In conclusion, S100A1B and S100BB dimers are increased in urine fluids collected from asphyxiated newborns who will develop HIE since first urination, and their measurement may be useful for the early detection, at

stages when monitoring procedures are still of no avail could be especially useful, of cases at risk.

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7. REFERENCES

1. Heizmann CW: Ca^{2+}-binding S100 proteins in the central nervous system. *Neurochem Res* 24:1097-1100 (1999)

2. Donato R: S100: a multigenic family of calcium-modulated proteins of the EF-hand type with intracellular and extracellular functional roles. *Int J Biochem Cell Biol* 33:637-68 (2001)

3. Fanò G, S. Biocca, S. Fulle, M.A. Mariggiò, S. Belia, P. Calissanpo: The S100: a protein family in search of a function. *Progr Neurobiol* 46:71-82 (1995)

4. Takahashi K, T. Isobe, Y. Ohtsuki, T. Akagi, H. Sonobe, T. Okuyama: Immunohistochemical study on the distribution of alpha and beta subunits of S100 protein in human neoplasm and normal tissues. *Virchows Arch B* 45:385-396 (1984)

5. Albuerne M, C.L. Mammola, F.J. Naves, B. Levanti, G. Germanà, J.A. Vega: Immunohistochemical localization of S100 proteins in dorsal root, sympathetic and enteric ganglia of several mammalian species, including man. *J Peripher Nerv Syst.* 3:243-53.(1998)

6. Hachem S, A. Aguirre, V. Vives, A. Marks, V. Gallo, C. Legraverend: Spatial and temporal expression of S100B in cells of oligodendrocyte lineage. *Glia* 51:81-97 (2005)

7. Haglid K.G, Q. Yang, A. Hamberger, S. Bergman, A. Widerberg, N. Danielsen: S100b stimulates neurite outgrowth in the rat sciatic nerve grafted with acellular muscle transplants. *Brain Res* 69:196-201 (1997)

8. Michetti F, D. Gazzolo: S100B protein in biological fluids: a tool for perinatal medicine. *Clin Chem* 48:2097-04 (2004)

9. Florio P, F. Michetti, M. Bruschettini, M. Lituania, P. Bruschettini, F.M. Severi, F. Petraglia, D. Gazzolo: Amniotic fluid S100B protein in mid-gestation and intrauterine fetal death. *Lancet* 364:270-2 (2004)

10. Gazzolo D, M. Bruschettini, V. Corvino, R. Oliva, R. Sarli, M. Lituania, P. Bruschettini, F. Michetti: S100B protein concentrations in amniotic fluid are correlated with gestational age and with cerebral ultrasound scanning parameters results in healthy fetuses. *Clin Chem* 47:954-6 (2001)

11. Gazzolo D, M. Lituania, M. Bruschettini, P. Bruschettini, F. Michetti: S100B protein concentrations in amniotic fluid are higher in monoamniotic than in diamniotic twins and singleton pregnancies. *Clin Chem* 49:997-9 (2003)

12. Gazzolo D, P. Vinesi, E. Marinoni, R. Di Iorio, M. Marras, M. Lituania, P. Bruschettini, F. Michetti: S100B protein concentrations in cord blood: correlations with gestational age in term and preterm deliveries *Clin Chem* 46:998-1000 (2000)

13. Gazzolo D, F. Michetti, M. Bruschettini, N. Marchese, M. Lituania, S. Mangraviti, E. Pedrazzi, P. Bruschettini: Pediatric concentrations of S100B protein in blood: age- and sex-related changes. *Clin Chem* 49:967-70 (2003)

14. Gazzolo D, M. Bruschettini, M. Lituania, G. Serra, E. Gandullia, F. Michetti: S100B protein concentrations in urine are correlated with gestational age in healthy preterm and term newborns. *Clin Chem* 47:6-7 (2001)

15. Jonsson H, P. Johnsson, P. Höglund, C. Alling, S. Blomquist: Elimination of S100B and renal function after cardiac surgery. *J Cardiothorac Vasc Anesth* 14:698-01 (2000)

16. Nelson KB, J.H. Ellenberg: Obstetric complications as risk factors for cerebral palsy or seizure disorders. *JAMA* 251:1843-1848 (1984)

17. Hankins GD, M. Speer: Defining the pathogenesis and pathophysiology of neonatal encephalopathy and cerebral palsy. *Obstet Gynecol*. 102: 628-36 (2003)

18. Nagdyman N, W. Komen, H.K. Ko, C. Muller, M. Obladen: Early biochemical indicators of hypoxic-ischemic encephalopathy after birth asphyxia. *Pediatr Res* 49: 502-6 (2001)

19. Counsell SJ, M.A. Rutherford, F.M. Cowan, A.D. Edwards: Magnetic resonance imaging of preterm brain injury. *Arch Dis Child Fetal Neonatal Ed.* 88:269-74 (2003)

20. ACOG Committee Opinion: Inappropriate use of the terms fetal distress and birth asphyxia. *Int J Gynecol Obstet* 61:309-10 (1998)

21. Levene MI, D.J. Evans, S. Mason, J. Brown: An international network for evaluating neuroprotective therapy after severe birth asphyxia. *Semin Perinatol.* 23: 226-33 (1999)

22. Sarnat HB, M.S. Sarnat: Neonatal encephalopathy following fetal distress: a clinical and electroencephalographic study. *Arch Neurol* 33:696-705 (1976)

23. HFR Prechtl: Assessment methods for the newborn infant: a critical evaluation. In: Stratton D, ed. Psychobiology of human newborn. Chichester, UK: Wiley, (1982)

24. Stephan C, S. Wesseling, T. Schink, K. Jung: Comparison of eight computer programs for receiver-

operating characteristic analysis. *Clin Chem.* 49:433-9 (2003)

25. Gazzolo D, R. Frulio, A. Roletti, P. Bruschettini, M. Lituania, F. Michetti: S100A1B and S100BB urine levels in preterm and term healthy newborns. *Clin Chim Acta*. 384: 186-7. (2007)

26. Anderson RE, A. Winnerkvist, L.O. Hansson, O. Nilsson, L. Rosengren, G. Settergren, J. Vaage: Biochemical markers of cerebrospinal ischemia after repair of aneurysms of the descending and thoracoabdominal aorta. *J Cardiothorac Vasc Anesth.* 17: 598-603 (2003)

27. Nylén K, M. Ost, L.Z. Csajbok, I. Nilsson, C. Hall, K. Blennow, B. Nellgård, L. Rosengren: Serum levels of S100B, S100A1B and S100BB are all related to outcome after severe traumatic brain injury. *Acta Neurochir (Wien).* 150: 221-7 (2008)

28. Anderson RE, L.O. Hansson, J. Liska: Increase in serum S100A1B and S100BB during cardiac surgery arises from extracerebral sources. *Ann. Thor. Surg*. 71:1512-17 (2001)

29. Nygren De Boussard C, P. Fredman, A. Lundin, K. Andersson, G. Edman, J. Borg: S100 in mild traumatic brain injury. *Brain Inj*. 18: 671-83 (2004)

Abbreviations: HIE: hypoxic ischemic encephalopathy; CNS: central nervous system; NICU: neonatal intensive care unit

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