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Perinatal asphyxia: Kidney failure does not affect S100B urine concentrations

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ABSTRACT

Background: S100B protein is a well-established marker of brain damage. Its importance in urine assessment is the convenience of a collection and sampling procedure that can be repeated without risk for the newborn. Since S100B is mainly eliminated by the kidneys and perinatal asphyxia (PA) is often associated with kidney failure we investigated whether S100B release might be kidney-mediated, thereby modifying the protein's reliability as a brain-damage marker.
Methods: We examined a cohort of healthy (n = 432) and asphyxiated newborns (n = 32) in whom kidney function parameters (blood urea and creatinine concentrations and urine gravity) and urine S100B concentrations were assessed in the first hours after birth. Data were analyzed by multiple logistic regression analysis with S100B as independent variable among a variety of clinical and laboratory monitoring parameters.
Results: S100B urine concentrations were significantly higher ($P<0.01$) in PA newborns than controls. No significant correlations ($P>0.05$, for all) between total urine S100B levels and kidney function parameters such as creatinine ($r=0.03$), urea ($r=0.04$) and urine gravity ($r=0.06$) were found. Multiple logistic regression analysis of a series of clinical and laboratory monitoring parameters (odds ratio at sampling: 9.47) with S100B as independent variable showed a positive significant correlation only between S100B levels ($P<0.001$) and the occurrence of PA.
Conclusion: The present study shows that altered kidney function is not an adverse and/or confounding factor in urine S100B assessment and marks a new step towards the introduction of longitudinal monitoring of brain constituents in clinical practice.

1. Introduction

Perinatal asphyxia (PA) and its major complication, hypoxic–ischemic encephalopathy (HIE), are important causes of mortality and morbidity in full-term newborns [1,2]. Despite accurate postnatal monitoring procedures, neurological handicap can occur in about 25–28% of these infants. The early post-asphyxia period is crucial because radiological findings may still be negative and brain damage at a subclinical stage [1–4]. In this setting, the measurement of quantitative parameters, such as constituents of the nervous tissue, able to detect subclinical lesions when routine monitoring procedures are still silent, could be particularly useful.

Among biochemical markers of brain damage, S100B is an acidic calcium-binding protein of the EF-hand family characterized by the common helix-loop-helix motif and concentrated mainly in the central nervous system (CNS). The protein has a half-life of about 1 h and is eliminated by the kidneys (about 98%) [5,6]. Elevated S100B concentrations in different biological fluids (i.e., cerebrospinal fluid, peripheral and cord blood) are a reliable marker of brain damage in adults, infants and fetuses [5,7–12]. Abnormal S100B levels in urine have also been found in the early postnatal period in newborns complicated by severe brain complications after acute and chronic hypoxia, such as intraventricular hemorrhage, HIE and ominous outcome [13–18]. Data on urine assessment support the expedience of the clinical

Abbreviations: PA, Perinatal asphyxia; HIE, hypoxic–ischemic encephalopathy; CNS, central nervous system; RBC, red blood cell count; SNAP-PE, Score for Neonatal Acute Physiology-Perinatal Extension; CI, Coefficient Intervals; BBB, brain blood barrier.

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use of S100B in longitudinal monitoring of high-risk newborns [13–18]. Additional advantages lie in the convenience of a fluid collection and sampling procedure that can be repeated without risk for the newborn [7,13–18]. However, bearing in mind that: i) S100B is eliminated mainly by the kidneys [6] and; ii) PA is often associated with kidney failure, the possibility that the release in urine of the protein could be affected by kidney function is an issue that needs to be elucidated.

The purpose of the present study therefore was to investigate, in a cohort of healthy and asphyxiated newborns in whom kidney function parameters (i.e. blood urea and creatinine concentrations and urine gravity) and urine S100B concentrations were assessed in the first hours after birth, whether the release of S100B in urine was kidney-mediated, thereby modifying the reliability of S100B as a marker of brain damage.

2. Materials and methods

2.1. Population

Between January 2001 and December 2005 we performed a cross-sectional study with urine samples collected consecutively at our tertiary referral centers for Neonatal Intensive Care Units (NICUs) from 480 term newborns (37–42 weeks of gestation; mean 39 weeks), of whom 432 were healthy and the remaining 48 complicated by PA and HIE.

Appropriate growth was defined by the presence of ultrasonographic signs (when biparietal diameter and abdominal circumference were between the 10th and the 90th centiles) according to the normograms of Campbell and Thoms [19] and by post-natal confirmation of a birth weight between the 10th and 90th centiles, according to our population standards and after corrections for the mother's height, weight and parity and the sex of the newborn. All healthy newborns admitted to the study fulfilled the following criteria: no maternal illness, no signs of fetal distress, pH>7.2 in cord blood or venous blood, Apgar scores at 1st and 5th minutes >7. We also included newborns who developed jaundice and required hospitalization for 96 h during which blood sampling was performed for bilirubin assessment, blood pH (including ion concentrations) and kidney function parameters. All newborns were in normal clinical conditions and showed no overt neurological syndrome on discharge from hospital (Table 1).

All asphyxiated newborns were delivered by emergency cesarean section on account of 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). Asphyxia was defined according to an Apgar score <3 at the 5th minute, pH <7.0, or BE <−12 in cord blood or venous blood taken from newborns within 60 min after birth, or the need for positive pressure ventilation (>3 min) [20]. Infants who met 3 or more of the above clinical and biochemical parameters were included in the asphyxia group. All patients had a normal karyotype and were free of detectable anomalies; fetuses with malformations or congenital heart disease and those born to women exposed to alcohol or tobacco smoke were excluded from the study. Other exclusion criteria were congenital or perinatal infections including chorioamnionitis, intrauterine growth retardation, multiple pregnancies, maternal drug addiction.

On admission to the NICU we routinely assessed laboratory parameters such as red blood cell count (RBC), venous blood pH, ion concentrations, plasma glucose, urea and creatinine levels and urine gravity. Neurological examination was performed daily [21]. S100B protein levels were measured at the same time as blood sampling. Results were correlated with kidney function parameters.

Table 1
Perinatal outcomes in asphyxiated newborns (PA) and in healthy subjects (controls). Values are expressed as mean ± SD.

	PA group (n = 48)	Controls (n = 432)
Perinatal clinical characteristics		
Birth weight — g	3342 ± 221	3407 ± 114
Gestational age >36 weeks — no.	48	432
Gender — male/female	23/25	216/210
Cesarean section — no./total	48/48*	120/432
Factors associated with primary outcomes		
Apgar score — no./total		
At 1 min <3	48/48*	0/432
At 5 min <3	48/48*	0/432
Respiratory distress syndrome — no./total	21/48*	0/432
Mechanical ventilation support — no/total	21/48*	0/432
Inotrope therapy — no./total-	31/48*	0/432
No-mild HIE	23/48*	0/432
Severe HIE	25/48*	0/432

* P<0.05 vs controls.

2.2. Neurological examination

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 h 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 the first 5 days from birth. 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 [21], 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 was 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. Cerebral ultrasound and neurological patterns were assessed at the same time as urine sampling by a single examiner in each Center who did not know the results of the urine test. Finally, the severity of illness in the first 24 h after birth was measured using the Score for Neonatal Acute Physiology-Perinatal Extension (SNAP-PE) [23].

2.3. S100B measurements

Urine samples were collected in the first hours after birth and centrifuged immediately at 900 *g* for 10 min; the resulting sera were stored at −70 °C. Urine S100B concentrations were measured by an immunoluminometric assay (Lia-mat Sangtec 100; AB Sangtec Medical), according to the manufacturer's instructions. Those who performed the index tests were blind to the results of other tests. The assay detection limit was 0.02 μg/L, the intraassay CV was ≤5.0%, and the interassay CV was ≤10%. The assay is specific for S100B, having been assessed by the manufacturer for the absence of cross-reactivity with other proteins of the S100 family.

3. Statistical analysis

The Kolmogorov–Smirnov test was used to evaluate whether the distribution of data was Gaussian. Comparison between proportions was performed using Fisher's exact test. S100B levels in urine are given as median, lower and upper 95% Coefficient Intervals (CI). Comparison of fetal and neonatal monitoring parameters between groups was performed by the Mann–Whitney *U*-test, Kruskal–Wallis one way ANOVA, followed by post-hoc Dunn's test when the data were not normally distributed. Correlations between S100B and kidney