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Protein S100B in umbilical cord blood as a potential biomarker of hypoxic-ischemic encephalopathy in asphyxiated newborns

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ABSTRACT

Background: Neonatal hypoxic ischemic encephalopathy (HIE) is a devastating condition resulting from a sustained lack of oxygen during birth. The interest in identifying a relevant biomarker of HIE has thrown into limelight the role of protein S100B as a clinical diagnostic marker of hypoxic brain damage in neonates.
Aims: To evaluate the diagnostic value of protein S100B, measured in umbilical cord blood immediately after birth, as a useful biomarker in the diagnosis of HIE Sarnat stages II-III as well as a marker for long-term mortality and morbidity.
Study design: Protein S100B was analyzed in cord blood sampled at birth from 13 newborns later diagnosed with stage II-III HIE and compared with 21 healthy controls. S100B concentrations were related to cord artery pH, amplitude-integrated electroencephalography (aEEG), stage of HIE, and death/sequelae up to an age of 6 years. Both parametric and non-parametric statistics were used with a two-sided $P < 0.05$ considered significant.
Results: The difference in S100B concentration was marginally statistically significant between HIE cases and controls ($P = 0.056$). Cord blood acidosis ($P = 0.046$), aEEG pattern severity ($P = 0.030$), HIE severity ($P = 0.027$), and condition at 6-year follow-up (healthy/permanent sequelae/death; $P = 0.027$) were all related to an increase in S100B concentration.
Conclusions: Protein S100B in neonates suffering from HIE stages II-III appeared elevated in umbilical cord blood at birth. The S100B concentrations were positively associated to the severity of disease and the risk of suffering from neurodevelopmental sequelae and even death.

1. Introduction

The fetal brain is vulnerable to severe and sustained hypoxia during birth, which can lead to hypoxic-ischemic encephalopathy (HIE). HIE is characterized by clinical and laboratory evidence of acute or subacute brain injury [1]. At the cellular level, the neuronal injury develops in two phases: an initial ischemic phase characterized by cell death within areas of poor blood perfusion, and a reperfusion phase after 2–6 h with apoptotic cell death and extension of the affected areas [2,3]. The dreary prognosis of neonates suffering from severe HIE is well documented [4–9].

However, if neuroprotective therapies are started before the reperfusion phase is fully developed, the extent of neuronal brain injury can be limited [10,11]. Thus, the very first few hours after birth represent a critical time window to identify candidates for treatment [12,13]. This has led to a search for biological markers that could early in the course help in identifying neonates at risk of developing HIE.

Several biomarkers have been studied, including protein S100B. S100B is released mainly from glial cells in response to neuronal injury [14] and is detectable in a variety of biological fluids such as urine, cerebrospinal fluid, blood and amniotic fluid [15,16]. Studies have shown associations between the severity of neuronal injury and the concentration of S100B [17–26], which has led to special interest in S100B in asphyxiated newborns. However, there have only been a few studies investigating brain injury biomarkers from umbilical cord blood sampled at birth [27].

Severe HIE is a rare condition affecting < 3–5 newborns per 1000 births per year [28] and collecting ample clinical material for studies on perinatal asphyxia is therefore challenging. We have access to a serum bank started several decades ago, where cord blood samples have been

Abbreviations: aEEG, Amplitude-integrated electroencephalopgraphy; ECLIA, Electro chemiluminiscent immunoassays; EEG, Electroencephalography; Hb, Hemoglobin; HIE, Hypoxic-ischemic encephalopathy; ICD, International Classification of Diseases; K-W test, Kruskal-Wallis test; M-W test, Mann-Whitney *U* test; SD, Standard deviation; S100B, S100 protein including the subunits αβ and ββ

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routinely collected at birth and stored frozen. We could thus perform a retrospective case-control study of cord S100B concentrations taken from newborns developing HIE Sarnat stages II-III [29] and compare them with healthy controls.

The primary aim of the study was to compare cord S100B concentrations between neonates with HIE stages II-III and healthy controls. We hypothesized that HIE II-III would correspond to higher cord S100B concentrations. A second aim of the study was to investigate the relationship between S100B concentrations and the grade of morbidity of affected infants, i.e., by stage of disease (HIE II-III), cord blood acidemia, amplitude-integrated electroencephalography (aEEG) abnormality, and long-term sequelae.

2. Material and methods

2.1. Retrieval of HIE cases

We searched five regional and local obstetric, neonatal and rehabilitation medicine databases for neonates with HIE born between 1990 and 2001 at the maternity unit of Skåne University Hospital in Malmö, Sweden. Diagnosis codes for infants suffering from HIE were identified using the International Classification of Diseases (ICD) 9th revision (ICD-9, Swedish version ICD-9-SE) as follows: 768E (fetal distress, unspecified as to time of onset, in liveborn infant), 768F (severe birth asphyxia), 779A (convulsions in newborn), and 779B (cerebral depression, coma, and other abnormal cerebral signs). We also searched for ICD-10-SE codes P90.9A (convulsions in newborn, verified by EEG), P91.0B (moderate HIE), P91.0C (severe HIE) and P21.1C (severe birth asphyxia).

Medical journals of the infants that matched the ICD codes were evaluated by a senior neonatologist (Author 2) to determine if they met the diagnostic criteria for HIE stage II or III according to Sarnat & Sarnat [29]. HIE stage II cases were categorized as lethargic with muscle hypotonia, overactive stretch reflexes, seizures, decreased suck and Moro reflexes and miotic pupils. HIE stage III cases were categorized as stuporous and flaccid with absent stretch, suck or Moro reflexes, mydriatic pupils with no reaction to light stimulus, no breathing and seizures absent or to some lesser extent. All correctly identified HIE infants that survived were regularly followed at pediatric and/or rehabilitation outpatient clinics until the age of at least 6 years.

2.2. Selection of controls

Using the maternity unit logbooks, each HIE case was matched to four controls with similar gestational age and neonate gender. Two controls born close before the matching HIE case and two born soon after were chosen. The first two controls that were found with the appropriate amount of umbilical cord sera stored frozen in the serum bank were selected as the final controls. Thus, each HIE case was matched with two controls. Mode of delivery was not matched for.

2.3. Retrieval of clinical data

Clinical data was retrieved from the pediatric and rehabilitation medical journals in HIE cases. Data about pregnancy progress, birth and perinatal outcome was retrieved from the local obstetric database for both cases and controls.

2.4. Umbilical cord serum bank samples

The maternity unit at Skåne University Hospital in Malmö has routinely collected both maternal venous and umbilical cord blood between the years 1969 to 2001. Mixed arterial and venous cord blood was collected by passive drainage into test tubes. The test tubes were refrigerated overnight at + 8 °C for sedimentation. The supernatant serum was collected the following morning and frozen in polypropylene plastic test tubes at − 20 °C until analysis.

2.5. Assessment of severity of adverse outcome

To test the hypotheses, we made the following comparisons between cases and controls: umbilical cord arterial pH at birth, aEEG classification, stage of HIE, and death or permanent sequelae up to an age of at least 6 years. The definition and diagnostic criteria for cerebral palsy were used according to international guidelines [30] and according to the national Swedish quality register for cerebral palsy follow-up [31].

2.6. Umbilical cord artery pH

Since 1981, umbilical cord arterial and venous blood gases have been routinely performed at birth at the Skåne University Hospital in Malmö. Cord blood acidemia was defined as a cord artery pH below a gestational age-adjusted mean value minus 2 standard deviations (SD) [32]. A pH < 7.00 [33] was also noted.

2.7. aEEG classification

The aEEG was classified according to Toet et al. [34]:

1) Continuous normal voltage (no periods of low amplitude).
2) Discontinuous normal voltage (low amplitude periods lasting < 10 s).
3) Burst-suppression.
4) Continuous extremely low voltage.
5) Flat tracing (isoelectric).

Traces with continuous or discontinuous normal voltage patterns and no abnormal changes were classified as normal; burst-suppression, continuous extremely low voltage and flat tracing were classified as abnormal [34]. The classification also took into consideration epileptic activity (single or repeated seizures, saw-tooth pattern, status epilepticus): a single seizure not confirmed by aEEG and not requiring antiepileptic treatment was regarded as benign. Thus, neonates with normal background activity aEEG were classified as having normal aEEG patterns.

2.8. Laboratory methods

The cord serum samples were vortexed and centrifuged at 2000*g* for 10 min according to standardized protocols accredited to the Department of Laboratory Medicine, Skåne University Hospital in Malmö. A volume of 130 μL of cord serum was transferred to standardized tubes for analysis. All cord serum samples from both cases and controls were analyzed in the same batch.

The samples were analyzed by a one-step immunometric sandwich-method with electrochemiluminiscence immunoassay (ECLI) (Elecsys S100, ref. 03175243190, Mannheim, Germany) using Cobas analyzer 6000 system with e601/e602 modules (Roche Diagnostics International, Rotkreuz, Switzerland). This method uses an antigen-antibody reaction resulting in emission of light, where the light intensity is directly proportional to the concentration of S100B [35]. The instrument detects S100B concentrations from 0.005 to 39 μg/L, with a coefficient of variation of 4%. S100B is an intracellular protein, where hemolysis with free hemoglobin (Hb) concentration > 10 g/L could influence the analysis [35].

To exclude the effect of hemolysis, free Hb in samples was determined by mixing 10 μL serum with 100 μL 0.01% Na_2CO_3 in a half well microplate reader (UV-star, Greiner Bio-One, Frickenhausen, Germany). After centrifugation for 1 min at 900*g*, absorbance was noted at 405, 450 and 380 nm. The Hb concentration was then calculated according to the Harboe eq. [36] and corrected for shorter light path-length in the microplate.