PREDICTING OUTCOME AFTER SEVERE BRAIN INJURY IN RISK NEONATES USING THE SERUM S100B BIOMARKER: RESULTS USING SINGLE (24h) TIME-POINT

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Abstract: Background and objectives: In recent years, biochemical markers have been employed to predict the outcome of risk neonates with severe asphyxia contributing to traumatic brain injury (TBI). In mild TBI S100B has shown the most promise as a marker of outcome. The objectives of this study were: (i) to show the range of serum S100B levels during the acute phase after asphyxia in neonates and premature newborns, (ii) to determine if S100B has potential to discriminate favourable from unfavourable outcomes in neonates and premature newborns with similar severity of brain injury and (iii) to establish an S100B ‘cut-off’ point for lethal outcome.

Methods: 119 neonates were recruited, divided into an overall risk group (N = 71) and control group (N = 48). The risk neonates were categorized into subgroups according to their clinical presentation. A serum blood sample was obtained from each patient at a 24 h post-injury time-point. S100B levels were measured using the ECLIA (Electro-Chemi-Luminiscence Immuno Assay) method. Injuries were coded using an internationally recognised injury severity scoring system (ISS).

Results: S100B levels were significantly higher in asphyxiated term neonates (N = 29; M = 0.64) than in premature neonates (N = 30; M = 0.18) and IUGR (intrauterine growth retardation) neonates (N = 9; M = 0.03). The neonates with a neurological defect (N = 3; M = 1.73) measured the highest level of S100B. The average serum S100B levels for the control group (N = 48) was 0.12 μgL(-1); cut-off point.
Conclusion: During the first 24h of life S100B protein in term neonates was significantly higher compared to all the other groups (cut-off was 0.12 μgL), except the neonates with neurological defects. S100B protein is a good indicator of brain damage in term neonates, especially in the first 24 h of life.

Key words: S100B protein, brain injury, risk infants, asphyxiated term neonates, cut-off point.

Introduction

The diagnosis of perinatal insults currently relies on adequate documentation of general medical and obstetric factors and on radiological and laboratory assessments. The measurement of brain constituents, such as S100B protein, may offer an alternative and direct indicator of cell damage in the nervous system when clinical and radiological assessments are still silent, and have the additional advantage of providing a quantitative indicator of the extent of brain lesions. S100B protein has been measured by several immunoassays in biological fluids (i.e. cerebrospinal fluid, blood, amniotic fluid and urine) from foetuses and newborns at high risk of perinatal brain damage. S100B protein in biological fluids increases at an early stage. S100B concentration has also been significantly correlated with the extent of brain lesions. S100B protein appears to satisfy the criteria for a brain injury marker in perinatal medicine: (a) simple to perform measurements with good reproducibility; (b) detection in a variety of biological fluids, possibly reducing perinatal stress related to testing; (c) possible use in longitudinal monitoring because of its 1-hour-long half-life; and (d) well-established use as an early and quantitative marker of brain lesions/damage. Finally, because of the neurotrophic role putatively played by S100B, its measurement in biological fluids at pre-/perinatal ages makes it a candidate for laboratory evaluation of brain maturation [1].

Actually, S100B protein reflects CNS injury. The excellent sensitivity of S100B has enabled it to confirm the existence of subtle brain injury in patients with mild head trauma, stroke, and after successful resuscitation from cardiopulmonary arrest. The extent of S100B elevation has been found to be useful in predicting the clinical outcome after brain injury. Elevation of S100B above certain threshold levels might be able to reliably predict brain death or mortality. A normal S100B level reliably predicts the absence of significant CNS injury. S100B protein can more reliably predict the extent of brain injury in clinical outcomes. In future, S100B measurements might reliably predict secondary brain injury and enable physicians to initiate therapeutic interventions in a timely manner. S100B levels have been shown to rise from several hours to a few days before changes in ICP, neurological examinations and neuro-imaging tests show up. S100B levels may also be used to monitor the efficacy of treatments [2].
Early assessment of the severity of the injury and the consequent prognosis are of major concern for physicians treating patients suffering from TBI (Traumatic brain injury). A reliable indicator to accurately determine the extent of the brain damage has to meet certain requirements: (i) origin in the central nervous system (CNS) with no contribution from extra-cerebral sources; (ii) a passive release from damaged neurons and/or glial cells without any stimulated active release; (iii) a lack of specific effects on neurons and/or glial cells interfering with the initial injury; (iv) an unlimited passage through the blood-brain barrier (BBB). The measurement of putative biochemical markers, such as the S100B protein, has been proposed for this role. Over the past decade, numerous studies have reported a positive correlation of S100B serum levels with a poor outcome following TBI. However, some studies raise doubt whether the serum measurement of S100B is a valid biochemical marker of brain damage. Recent experimental findings suggest a possible therapeutic potential of S100B [3]. S100B is a calcium-binding peptide and is used as a parameter of glial activation and/or glial death in many disorders of the CNS. It plays an important role in normal CNS development and recovery after brain injury. Although S100B is mainly found in astroglial and Schwann cells, it also has extra-cerebral sources. S100B is a useful neuro-biochemical marker of brain damage, such as in circulatory arrest, stroke and TBI. S100B is also associated with chronic neurological diseases [4].

Neonatal brain injury due to intra-partum asphyxia is an important cause of cerebral palsy (CP), mental retardation and epilepsy. Despite advances in perinatal care over the past three decades, the incidence of CP attributed to birth asphyxia has not changed. One reason is that we do not know specifically how to intervene in the postnatal period to prevent hypoxic-ischemic encephalopathy (HIE), which may follow intra-partum asphyxia and ultimately result in cerebral palsy. Nor do we know how to identify the neonates with asphyxia who are at greatest risk for encephalopathy and therefore are most likely to benefit from an intervention [5].

Perinatal hypoxic-ischaemic cerebral injury begins during an asphyxia insult, usually caused by an interruption in placental blood flow and gas exchange, and extends into a recovery period after resuscitation (the reperfusion interval). Subsequent tissue injury takes the form of either selective neuronal necrosis or apoptosis. At a cellular level, when cerebral perfusion is too low to provide adequate cerebral oxygenation, a cascade of biochemical events is initiated, including energy failure, acidosis, free radical formation, Ca accumulation, lipid peroxidation and neurotoxicity from glutamate and nitric oxide. These events disrupt cell structure and ultimately cause cell death. At the onset of reperfusion, several of these processes are further stimulated by the reintroduction of oxygen, so that injury continues. It is during this interval after resuscitation from HIE that an intervention to reduce the severity of ongoing brain damage might be efficient. In adults, the processes of neuronal necrosis and apoptosis are slow,
so this interval may last from several hours to a day or more [6]. In infants, however, cellular destruction is much more rapid, so that the interval during which an intervention might be effective is probably no longer than two to six hours after termination of the hypoxic-ischemic insult. Thus early identification of infants with asphyxia who are at highest risk of human brain injury is critical if reperfusion injury is to be prevented. The 24 h time point was chosen as a period in which we can easily affect the reperfusion phase, by involving hypothermia, standard intensive care treatment and/or mechanical ventilation.

Thus it is not surprising that common factors assessed during labour to identify fetuses with asphyxia that are at risk of brain injury, such as foetal heart-rate abnormalities and thick meconium staining of the amniotic fluid, rarely predict subsequent cerebral palsy. Similarly, a single postnatal marker used to identify high risk infants, such as a low Apgar score or need of resuscitation in the delivery room, also rarely predicts cerebral palsy.

Epidemiology of perinatal brain damage

Our knowledge of the timing of adverse insults is important in relation to future measures of prevention. However, such knowledge is still incomplete and debatable. For example, the reported contribution of asphyxia at birth to CP in infants born at term varies from 8% to 28% [7, 8, 9]. Our understanding of the timing of insults and of contributing factors may be improved by adequate documentation of general medical and obstetric factors, determination of pH and blood gases in cord blood, and neonatal neuro-imaging. Other diagnostic tools that may be of crucial importance are measurements of markers of perinatal brain injury in biological fluids. The key word is "prevention", with the aim of improving our ability to detect foetuses and newborns at risk of brain injury at an earlier stage, when the window for therapeutic action is still open [10]. The possibility of longitudinal monitoring of the effects of drugs and supportive care is second in importance only to prevention as a means of minimizing brain trauma and the number of neurologically handicapped children.

Biochemical, Biological, and Pathophysiological Features of S100B

The term S100B refers to members of a multigenic family of calcium-modulated proteins (S100 proteins), mostly of low molecular mass (10,000 Dalton) that were first identified (on the basis of methods available at the time) as a protein fraction detectable in the brain, but not in non-neural extracts. They are called S100 because of their solubility in a 100%-saturated solution with ammonium sulphate [11]. At present, at least 20 proteins have been identified as belonging to the S100 protein family, the members of which are characterized by the presence of a pair of so-called EF-hand (i.e., helix-loop-helix) calcium-binding motifs [12], first discovered in the crystal structure of parvalbumin [13], that induce conformational changes of the protein after binding to calcium [14, 15].
**S100B protein in Bio-fluids as a Marker of Brain Damage**

S100B protein has been measured in several biological fluids (CSF, blood, urine and amniotic fluid) by a series of immunoassays, which were indiscriminately used in various fluids. The earliest studies used immunoassays such as a microcomplement fixation assay, RIA (Radioimmunoassay) and a particle-counting immunoassay directly developed by the authors, whereas more recent studies have used very simple, sensitive and inexpensive commercially available immunoassays such as a two-site IRMA (Sangtec), an immunoluminometric assay (Sangtec), and an ELISA (SynX Pharma). PCR has also been used to analyse S100B in blood and amniotic fluid.

**Study aim**

The objectives of this study are:

a) to show the range of serum S100B levels during the acute phase after asphyxia

b) to determine if S100B has the potential to discriminate favourable from unfavourable outcomes in neonates and premature infants with similar severity of brain injury

c) to establish an S100B ‘cut-off’ predictive for death.

**Methods**

One hundred and nineteen (119) neonates were recruited in total. All risk neonates with severe asphyxia admitted to the Neonatal and Paediatric Intensive Care Unit at the University Pediatric Hospital in Skopje within 24 h of injury were eligible for inclusion in the study. One serum blood sample was obtained from each patient at the 24 h post-injury time-point. S100B levels were measured using the ECLIA (Electro-Chemi-Luminiscence Immuno Assay) method – Elecsys 2010-Roche Diagnostic. Injuries were coded using an internationally recognised injury severity scoring system (ISS).

**Results and discussion**

To analyse the differences among the groups we used single factor ANOVA, followed by the Games-Howell post-hoc test to determine specifically the statistical significance of each difference. The statistical indicators for the levels of measured S100B at a 24 h post-injury time-point in each group are presented in Table 1.
Table 1

**Descriptive statistics of S100B analysed levels during first day of admission among all the groups**

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Std. Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>48</td>
<td>0.119</td>
<td>0.050</td>
<td>0.007</td>
</tr>
<tr>
<td>Asphyxiated term neonates</td>
<td>29</td>
<td>0.642</td>
<td>0.352</td>
<td>0.065</td>
</tr>
<tr>
<td>Asphyxiated Premature neonates</td>
<td>30</td>
<td>0.183</td>
<td>0.250</td>
<td>0.046</td>
</tr>
<tr>
<td>Asphyxiated Neonates with IUGR</td>
<td>9</td>
<td>0.029</td>
<td>0.020</td>
<td>0.006</td>
</tr>
<tr>
<td>Asphyxiated Neonates with ND</td>
<td>3</td>
<td>1.733</td>
<td>1.086</td>
<td>0.627</td>
</tr>
</tbody>
</table>

The average serum S100B level for the control group (N = 48) was 0.12 µgL(-1) (cut-off point). S100B levels were higher in asphyxiated term neonates (N = 29; M = 0.64 µgL) than in the control group, in premature neonates (N = 30; M = 0.18 µgL) and neonates with IUGR (N = 9; M = 0.03 µgL), with the exception of the neonates with a neurological defect (N = 3; M = 1.73 µgL).

**Diagram 1 – Average levels of S100B protein for each group at 24 h after birth**

From the diagram we can see that all the risk groups showed S100B values above the cut-off point, except for the neonates with a neurological defect. Also, we can see an extreme difference in the S100B serum levels between the neonates with IUGR (intra uterine growth retardation) and the neonates with ND (neurological defect), which is indicative of their neurological
condition, thus implying that S100B is more sensitive as a predictive biomarker in TBI.

The ANOVA test of difference between groups was statistically significant with F(4,114) = 44.73 at p < 0.001 (for details see Tables 2 and 3).

Table 2

<table>
<thead>
<tr>
<th></th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>12,201</td>
<td>4</td>
<td>3,050</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Within Groups</td>
<td>7,774</td>
<td>114</td>
<td>0.068</td>
<td>44.730</td>
<td>0.000</td>
</tr>
<tr>
<td>Total</td>
<td>19,974</td>
<td>118</td>
<td></td>
<td>44.730</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Table 3

<table>
<thead>
<tr>
<th></th>
<th>Mean difference (I-J)</th>
<th>Control group</th>
<th>Asphyxiated term neonates</th>
<th>Asphyxiated Premature neonates</th>
<th>Asphyxiated Neonates with IUGR</th>
<th>Neonates with ND</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control group</td>
<td>– 0.523*</td>
<td>– 0.065</td>
<td>0.090*</td>
<td>– 1.614</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>Asphyxiated term</td>
<td>0.523*</td>
<td></td>
<td>0.459*</td>
<td>0.613*</td>
<td>– 1.091</td>
</tr>
<tr>
<td></td>
<td>neonates</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>Asphyxiated Premature</td>
<td>0.0647</td>
<td>– 0.459*</td>
<td>0.154**</td>
<td>– 1.550</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Neonates with IUGR</td>
<td>– 0.090*</td>
<td>– 0.613*</td>
<td>– 0.154**</td>
<td>– 1.704</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>Asphyxiated Neonates with ND</td>
<td>1.614</td>
<td>1.091</td>
<td>1.550</td>
<td>1.704</td>
<td></td>
</tr>
</tbody>
</table>

* p < 0.001  
** p < 0.05

From the results of the Games-Howell test we can confirm that the asphyxiated term neonates have significantly higher values of S100B measured at 24 h after birth in comparison to the control group and the asphyxiated pre-term neonates and asphyxiated neonates with IUGR. The neonates with a neurological defect measured the highest level of S100B but without any statistically significant difference compared to the rest of the groups.
Conclusion

S100 B protein in term neonates was significantly higher in the first 24 h after birth compared to all the other groups except the neonates with neurological defects, which showed higher levels of S100B protein with no statistical significance. S100B protein is a good indicator of brain damage in term neonates, especially in the first 24 h after birth.

REFERENCES

Предвидување на исходот по мозочно оштетување кај ризични новородени со користење на S100B протеински маркер: резултати со користење на еден временски период (24 ч)

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Вовед и цел: Во последните неколку години, биохемиските маркерњи се користат за предвидување на исходот на болеста кај ризични новородени со тешка асфиксија кои може да развијат трауматско мозочно страдање (TMC). Каж полесните случаи на TMC, S100B ветува најмногу како маркер за исходот на болеста. Целта на овој труд кај новородените со тешко новороден се: (i) да се прикаже опсегот на серумските вредности на S100B протеинот во текот на акутната фаза по периодот на асфиксия; (ii) утврдување дали S100B може да се користи за разликување на саканот од несаканот исход кај терминските и прематурните новородени со слична тежина на мозочно страдање и (iii) да се утврди гранична вредност на S100B која може да предвиде смртен исход.

Методи: Во истражувањето беа вклучени 119 новородени, поделени во две главни групи: ризична и контролна. Сите ризични новородени беа категоризирани во подгрупи според клиничката слика. Примерокот на серум беше земен од секој пациент 24 часа по повредата. Вредностите на S100B беа мерени со користење на ECLIA метода. Степенот на травма беше проценет според меѓународно познатиот систем за процена на тежината на повредата (ISS).

Резултати: Средната вредност на S100B во контролната група (N = 48) беше 0.12 microgL(-1) (гранична точка). Вредностите на S100B беа значително повисоки кај терминските новородени со асфиксия (N = 29; M = 0,64), во однос на прематурните новородени (N = 30; M = 0,18) и новородените со ИУЗР (интраутеринно заостанување во развојот) (N = 9; M = 0,03). Додека највисока вредност на S100B беше измерена кај новородените со невролошки дефект (N = 3; M = 1,73).

Заклучок: Во текот на првите 24 часа по раѓањето S100B протеинот кај терминските новородени е значително повисок во споредба со сите други групи, освен кај новородените со невролошки дефекти, чија група покажа статистички несигнификантно повисоки вредности на S100B протеинот. S100B протеинот е

Придони, Од. биол. мед. наук, XXXIII/1 (2012), 147-156
добар индикатор за мозочно страдање кај терминските новородени, особено во текот на првите 24 часа од животот.

Ключни зборови: S100B протеински маркер, мозочно оштетување, ризични новородени, асфиктични доносени, гранична вредност.

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