values, and overall accuracies will be observed in the diagnosis in acutely decompensated heart failure when compared with use of titrating treatment in established chronic heart failure (as in the current trial) or with population screening for asymptomatic ventricular impairment. All of these potential applications remain under investigation at present, and appropriate cut-points will emerge from large and varied cohorts that are beyond the scope of the current report.

The reason for the discordance between the two assays at higher concentrations cannot be conclusively defined from our current data. Our competitive RIA uses a single polyclonal antibody directed to the extreme amino-terminal region of N-BNP. This terminus is subject to some variation and is not observed with the Elecsys assay, which uses antibodies directed to possibly more stable epitopes. The RIA is calibrated with a truncated peptide comprising the first 21 amino acids of N-BNP 1–76, whereas the Elecsys assay uses the full 76-amino acid peptide, and this may potentially lead to increasing divergence of measured values over the range of the two assays. The Elecsys assay results agree with our previous findings, suggesting that it is a reliable assay and should allow routine use of plasma N-BNP assays worldwide.

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References

Maternal Glucocorticoid Supplementation and S100B Protein Concentrations in Cord Blood and Urine of Preterm Infants, Diego Gazzolo,1 Maria Kornacka,2 Matteo Bruschettini,1 Mario Lituania, 1 Lia Giovannini, 1 Giovanni Serra,1 Urszula Majewska,2 and Fabrizio Michetti2 (1 Department of Pediatrics and Obstetrics, G Gaslini Children’s University Hospital, I-16167 Genoa, Italy; 2 Department of Neonatology, Warsaw Medical University Hospital, P-00315 Warsaw, Poland; 3 Institute of Anatomy and Cell Biology, Catholic University, Largo Francesco Vito, 1, I-00168 Rome, Italy; * author for correspondence: fax 39-0630154813, e-mail fabrizio.michetti@rm.unicatt.it)

Maternal glucocorticoid (GC) supplementation is widely used for the prevention of lung immaturity (1, 2), but its possible harmful effects on other organs, including the central nervous system (CNS), are still a matter of debate (3–9).

S100B, which is present mainly in the nervous system (10) and has a short half-life (11), is regarded as a useful marker of brain injury, although at physiologic concentrations it may act as a cytokine with a neurotrophic effect (10, 12–19). S100B concentrations in cord blood or urine...
have already been used to monitor brain distress on the fetal/newborn CNS (15, 17–21).

We investigated the possible effects of maternal GC administration on the CNS of newborns by measuring S100B in cord blood and longitudinally in urine. Between April 2000 and June 2002, we measured S100B in the umbilical cord blood and urine of infants, born at our tertiary referral centers for obstetric care, who were admitted to the neonatal intensive care units (NICUs) of our hospitals. From our database we retrieved the 39 infants whose mothers had been treated antenatally with corticosteroids and matched them for gestational age at recording with 39 infants whose mothers had not been treated with steroids (1 control for each patient in the GC group). Exclusion criteria included multiple pregnancies, fetal/neonatal cardiac or hemolytic diseases, fetal malformations, and chromosomal abnormalities.

The mothers in the GC group had received betamethasone (12 mg intravenously for 2 days) when spontaneous or planned preterm delivery was expected to occur. The main complications in the cases admitted to the study were intrauterine growth retardation (14 of 39 in the GC group; 15 of 39 in the control group), pregnancy-induced hypertension (13 of 39 in the GC group; 14 of 39 in the control group), and preterm delivery not complicated by intrauterine growth retardation, maternal hypertension, or diabetes (12 of 39 in the GC group; 10 of 39 in the control group).

S100B protein was measured at delivery in the umbilical cord blood and subsequently in urine at five predetermined points: at first urination (time 0) and at 24 h (time 1), 48 h (time 2), 72 h (time 3), and 120 h of age (time 4).

On admission to the NICU, all newborns routinely had measurements of red blood cell count, venous blood pH, ion concentrations, plasma glucose, urea, creatinine clearance, osmolality, urinary specific gravity, and arterial blood pressure; all underwent cerebral ultrasound and neurologic examinations. For cases in which GC had been administered, clinical and laboratory tests and cerebral ultrasound scans recorded at the predetermined monitoring time points were reevaluated and compared with those obtained from a control group.

The local Ethics Committees approved the study, and the parents of the infants/patients gave informed and signed consent.

Standard cerebral ultrasound was performed by real-time ultrasound at the same time points as urine sampling, and the grade of intraventricular hemorrhage (IVH) was classified (22).

The neurologic condition of each neonate was classified qualitatively (23) as normal, suspect, or abnormal. An infant was considered as having an abnormal neurologic condition when hyper- or hypokinesia, hyper- or hypotonia, hemi syndrome, apathy syndrome, or hyper excitability syndrome was present. An infant was classified as suspect if isolated symptoms, but no defined syndromes, were present. The same examiner tested all of the infants.

At delivery, the umbilical cord was clamped before any signs of breathing were seen, and blood was drawn from the umbilical vein for S100B measurements. None of the patients who underwent cesarean section experienced uterine contractility before the surgical procedure (Table 1).

Blood and urine samples were centrifuged at 900g for 10 min, and the supernatants were stored at −70°C before measurement. S100B was measured by an immunoluminometric assay (Lia-mat Sangtec 100; AB Sangtec Medical) that specifically measures the β-subunit, which is known to be predominant in the human brain (17, 18, 24). The results reported are the means of duplicate measurements. The limit of detection of the assay (B0 + 3 SD) was 0.02 μg/L, and the intra- and interassay imprecisions (as CVs) were ±5.5% and ±10%, respectively, at 0.28–4.2 μg/L.

The data are reported as the mean (SD). Groups were compared by the Kruskal–Wallis one-way ANOVA or by Mann–Whitney U-test for non-gaussian-distributed data. The Fisher exact test was used for comparisons of the incidences of neonatal neurologic outcome in patient groups and of acute respiratory distress syndrome (RDS) in patients vs controls.

Fetal and neonatal characteristics are summarized in Table 1. The incidence of RDS, IVH, and neurologic abnormalities, the need for mechanical ventilation support, and NICU hospitalization duration were higher in the infants whose mothers had not received antenatal GC treatment (P < 0.05): 20 preterm control infants developed IVH grade I and 4 developed IVH grade II, whereas there were no cases of IVH in the GC group.

S100B measurements in cord blood showed no significant differences between the two groups (P > 0.05). In infants born to untreated women, mean blood S100B was significantly higher in group B [brain damage; 3.67 (1.20) μg/L] than in group A [no brain damage; 1.11 (0.24) μg/L] or in infants in the GC group [1.11 (0.24) μg/L; Table 1].

<table>
<thead>
<tr>
<th>Characteristics of infants studied.</th>
<th>Controls (n = 39)</th>
<th>GC (n = 39)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age, years</td>
<td>27 (2)</td>
<td>26 (3)</td>
</tr>
<tr>
<td>Cesarean section, n</td>
<td>15</td>
<td>13</td>
</tr>
<tr>
<td>Gestational age at birth, weeks</td>
<td>34 (2)</td>
<td>34 (1)</td>
</tr>
<tr>
<td>Birth weight, kg</td>
<td>1.69 (0.56)</td>
<td>1.72 (0.43)</td>
</tr>
<tr>
<td>M/F, n</td>
<td>16/13</td>
<td>17/12</td>
</tr>
<tr>
<td>Apgar, yes/total</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st min &lt;7</td>
<td>5/39</td>
<td>4/39</td>
</tr>
<tr>
<td>5th min &lt;7</td>
<td>0/39</td>
<td>0/39</td>
</tr>
<tr>
<td>RDS, yes/total</td>
<td>16/39</td>
<td>7/39</td>
</tr>
<tr>
<td>Neurologic abnormalities, normal/suspect/abnormal</td>
<td>8/14/17</td>
<td>20/19/0</td>
</tr>
<tr>
<td>Cerebral ultrasound, normal/IVH</td>
<td>15/24</td>
<td>39/0</td>
</tr>
<tr>
<td>Mechanical ventilation support, yes/total</td>
<td>22/39</td>
<td>9/39</td>
</tr>
<tr>
<td>Mean NICU stay, days</td>
<td>31</td>
<td>22</td>
</tr>
<tr>
<td>Sepsis (within 7 days from birth), yes/total</td>
<td>7/39</td>
<td>5/39</td>
</tr>
</tbody>
</table>

*Mean (SD).

*P < 0.05.
Cerebral bleeding

S100B has been shown to be an early indicator of risk for postnatal brain damage. The results for healthy preterm newborns whose mothers were not treated with GC are in agreement with previous observations of S100B in the urine of healthy preterm infants (18). The high concentrations of urinary S100B observed in brain-damaged newborns whose mothers were not treated with GC offer laboratory support for a previous observation (20).

The present research shows that a well-established biochemical marker of brain distress, S100B protein, is present in significantly lower concentrations in the urine of newborns whose mothers were treated antenatally with GC than in newborns whose mothers were not treated. Urinary S100B concentrations in infants born to GC-treated mothers were also lower than those in infants without brain damage born to untreated mothers when these were subgrouped according to the occurrence of postnatal brain damage. The results for healthy preterm newborns whose mothers were not treated with GC are in agreement with previous observations of S100B in the urine of healthy preterm infants (18). The high concentrations of urinary S100B observed in brain-damaged newborns whose mothers were not treated with GC offer laboratory support for a previous observation (20).

The presence of S100B in urine has already been demonstrated in healthy newborns, and increased urinary S100B has been shown to be an early indicator of risk for cerebral bleeding (20). Because we found no significant differences in renal function among the three groups studied, the lower S100B in the infants in the GC group is not likely to be attributable to different concentrations of the protein in urine. Furthermore, because S100B is absent from kidney tissue, it is reasonable to suppose that its source in the urine is the CNS (10, 17). On the other hand, it is possible that at least some S100B may be released from other sites in which it is concentrated, such as adipose tissue (25), although data in this setting are lacking.

The longitudinal low S100B concentrations in infants from GC-treated mothers are intriguing. One explanation lies in the lower incidence of neurologic abnormalities in the GC group; these abnormalities almost certainly have a relevant role in the release of the protein into the systemic circulation and the urine. Another explanation may reside in the fact that infants born of GC-treated mothers require fewer intensive care interventions that affect blood–brain barrier permeability (2) and would thus likely increase the release of S100B into the systemic circulation (26) and finally into the urine. In addition, it should be noted that data on experimental models and in humans support the hypothesis that antenatal GC administration decreases blood–brain barrier permeability, protecting the brain (27, 28).

S100B is regarded as a cytokine with a neurotrophic role at low concentrations and a neurotoxic effect at high concentrations (10, 11, 29). Our findings of lower S100B concentrations after GC administration open new possibilities for studies aimed at investigating the possible effects of GC on the activity of S100B as a cytokine. Because GCs are known to affect cytokine production in some conditions and GC receptors have been shown to be present in S100B-producing cell types, the possibility that GC action on the CNS is accompanied by a modulation of S100B production and/or release should be taken into consideration (30–32). The present finding of low S100B concentrations after maternal GC treatment appears to be relevant, especially for other perinatal conditions in which GC treatment is used, such as the prevention of bronchopulmonary dysplasia in preterm infants.

Data on S100B measurements in cord blood indicate that GC administration is associated with lower concentrations of the protein in infants not affected by brain injury, consistent with the data regarding urine. Another difference between urinary and cord blood S100B could be related to the possibility that at least a part of the protein present in cord blood has a placental origin, as supported by recent observations (33, 34).

In conclusion, the present findings offer additional data in the debate concerning the effects of antenatal GC administration on the fetal/newborn brain, suggest the use of S100B as a tool to assess the effects of antenatal drug treatment, and offer a clue for future studies on a possible GC-derived modulation of S100B.

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References


High Concentrations of Excised Oxidative DNA Lesions in Human Cerebrospinal Fluid, Rafal Rozalski,1 Piotr Winkler,2 Daniel Gackowski,1 Tomasz Paciorek,1 Heliodor Kasprazk,1 and Ryszard Olinski1,2 (1 Department of Clinical Biochemistry, The Ludwik Rydygier Medical University in Bydgoszcz, Karlowicza 24, 85-092 Bydgoszcz, Poland; 2 Clinic of Neurosurgery, The Ludwik Rydygier Medical University in Bydgoszcz, Sklodowskiej-Curie 9, 85-092 Bydgoszcz, Poland; *author for correspondence: fax 48-52-585-3771, e-mail ryszardo@aci.ambydgoszcz.pl)

The high rate of oxygen consumption per unit mass of tissue renders the brain especially vulnerable to the deleterious effects of oxidative stress, which can arise from the overproduction of reactive oxygen species or from a deficiency of the antioxidant defense systems. Reactive oxygen species have the potential to modify all four DNA bases. Production of 8-hydroxyguanine (8-OH-Gua) reflects one of the most critical lesions of this type (1–3).

Products of DNA damage repair are excreted into the urine or other extracellular fluids without further metabolism (4–6). The rates of excretion of 8-OH-Gua and 8-hydroxy-2′-deoxyguanosine (8-OH-dGuo; modified base and nucleoside, respectively) in urine may be useful indicators of oxidative DNA damage and reflective of overall oxidative stress (6). It is also likely that the concentrations of modified base and nucleoside in urine reflect the activities of different repair pathways responsible for the removal of 8-OH-Gua from DNA, i.e., base excision repair and nucleotide excision repair (NER) (7, 8), which produce, respectively, 8-OH-Gua and 8-OH- dGuo. The analysis of 8-OH-Gua in body fluids presents