High Maternal Blood S100B Concentrations in
Pregnancies Complicated by Intrauterine Growth
Restriction and Intraventricular Hemorrhage

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Background: Intrauterine growth restriction (IUGR) is
associated with perinatal mortality and with neurologic
damage from intraventricular hemorrhage (IVH). We
investigated whether S100B, a neural protein found in
high concentrations after cell injury in the nervous
system, is increased in serum of women whose pregnan-
cies are complicated by IUGR and whose newborns
develop IVH. We also explored the prognostic accuracy
of maternal serum S100B for IVH in the newborn.

Methods: We conducted a case–control study of 106
pregnancies complicated by IUGR, including a sub-
group (n/H1154926) who developed IVH after birth, and 212
unaffected pregnancies matched for gestational age.
Ultrasound examination, Doppler velocimetry patterns
(in the utero-placental vessels and middle cerebral ar-
tery), and maternal blood collection were performed
before birth; cerebral ultrasound and neurologic exam-
inations were performed after birth.

Results: S100B was higher (P < 0.001) in IUGR pregnan-
cies complicated by IVH than in those that were not and
in controls. At a cutoff of 0.72 μg/L, sensitivity was 100%
[95% confidence interval (95% CI), 87%–100%] and spec-
ificity was 99.3% (97.5%–99.9%) for prediction of IVH
(area under the ROC curve, 0.999). The prevalence of
IVH was 8.2% in the whole study population, 93% (95%
CI, 83.6%–100%) in those with maternal S100B >0.72
μg/L, and 0% (0%–2.5%) in those with maternal S100B
<0.72 μg/L.

Conclusion: For prediction of IVH, measurements of
maternal S100B may be useful at times before clinical,
laboratory, and ultrasound patterns can identify risk of
IVH.

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Intrauterine growth restriction (IUGR) is defined as
persistent suppression of genetic growth potential that
occurs in response to a decrease in substrate supply.
Despite relevant progress in obstetric clinical care, IUGR
is associated with increased perinatal mortality and ac-
counts for ~40% of neurologically damaged children (1).
Clinical evidence suggests that fetal preexposure to ad-
verse intrauterine conditions, such as the decreased oxy-
gen and substrate supplies that occur in IUGR, plays a
causal role in perinatal mortality and central nervous
system (CNS) injury, represented mainly by the oc-
currence of intraventricular hemorrhage (IVH) (1). The abil-
ity to monitor high-risk fetuses in the perinatal period by
use of biochemical indexes could be particularly useful in
detecting cases at risk of adverse neurologic outcomes, to
determine the timing of insults that cause damage CNS as
early as possible with respect to future measures of
prevention (2, 3).

S100B is an acidic calcium-binding protein found in the
glial cells, astrocytes, Schwann cells, and neurons; it is
thought to be involved in the regulation of several cellular

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functions (cell–cell communication, cell growth, cell structure, energy metabolism, contraction, and intracellular signal transduction) (4). The findings of increased S100B concentrations in biological fluids (e.g., cerebrospinal fluid, blood, urine, and amniotic fluid) of adults (5–7), infants (8, 9), and fetuses (10–15) after cell injury in the nervous system have supported the use of S100B as a biochemical marker of brain damage (16).

In the present study, we investigated whether maternal serum S100B concentrations are increased in pregnancies complicated by IUGR and IVH and calculated the sensitivity and specificity of the measurement of maternal serum S100B for predicting the occurrence of IVH.

**Materials and Methods**

**Participants**
Informed consent was obtained from all women before inclusion in the study, and approval was obtained from our local human investigation committees.

A gestational age–matched case–control study was conducted (from January 2001 to July 2004) at our tertiary referral centers for obstetric care. The study group included singleton pregnancies complicated by IUGR (n = 106; gestational age range, 28–38 weeks), as defined by the presence of ultrasonographic signs (biparietal diameter below the 10th centile and abdominal circumference below the 5th percentile), according to the normograms of Campbell and Thoms (17) and by postnatal confirmation by a birth weight below the 10th percentile according to our population standards after corrections for mother’s height, weight, and parity and the sex of the newborn (18). A subset of IUGR fetuses (n = 90) were delivered by emergency cesarean section within 24 h after the last Doppler study; their indications included placenta abruption and non-reassuring fetal status according to the American College of Obstetricians and Gynecologists (bradycardia, fetal heart rate showing late decelerations, severe and repetitive fetal heart rate with variable decelerations, decreased beat-to-beat variability) (19). Fetuses with any malformation or cardiac or hemolytic disease were excluded from the study. Other exclusion criteria were multiple pregnancies, gestational diabetes, and any maternal CNS illness. The control group included 212 newborns from women with uncomplicated pregnancies (2 controls for each IUGR case) with a birth weight between the 10th and 90th percentiles according to gestational age, a normal postnatal neurologic outcome at the 7th day after birth, and fulfillment of all of the following criteria: no maternal illness; no signs of fetal distress; pH >7.2 in cord or venous blood; and Apgar scores >7 at 1 and 5 min. In all cases, gestational age was determined by the last menstrual period and confirmed by a first-trimester ultrasound scan.

**Fetal-placental Doppler Findings**
The flow velocity waveforms (FVWs) in the umbilical artery (UA) and the fetal middle cerebral artery (MCA) were recorded in all pregnancies in both the case and control groups within 24 h from delivery by means of a duplex pulsed color Doppler ultrasound (Aloka; SSD-2000) with a convex 3.5-MHz transducer. The pulsatility index (PI), defined as the (peak systolic velocity − end diastolic velocity)/mean velocity, was calculated automatically by the built-in software. A spatial peak temporal average <100 mW/cm² was used for blood flow measurements in the MCA. A 100-Hz high-pass filter was used, and Doppler waveforms were obtained in the absence of fetal body or breathing movements. In every record, 3 to 5 consecutive cardiac cycles were examined, and a mean of at least 3 values from each vessel was used for subsequent analyses. An abnormal PI for the UA was defined as above the 95th centile for gestational age for uncomplicated pregnancies. Similarly, an abnormal MCA PI below the 5th centile for gestational age for uncomplicated pregnancies and UA PI/MCA PI ratio >1 were considered as indexes of fetal compensatory mechanism in response to hypoxia (the so-called brain-sparing effect) (20).

**Cranial Assessments**
Cerebral ultrasound scanning was performed routinely in IUGR newborns within the first 72 h and at day 7 after birth by use of a real-time ultrasound instrument (Acuson; 128SP5), with a transducer frequency emission of 3.5 MHz. In the controls, cerebral ultrasound patterns were evaluated before discharge from the hospital.

After IVH was diagnosed according to Papile et al. (21), and based on the presence or absence of brain sparing, the IUGR group was subdivided as follows: group A (n = 46), no brain-sparing effect and normal cerebral ultrasound patterns; group B (n = 34), brain-sparing effect and normal cerebral ultrasound patterns; group C (n = 26), brain-sparing effect and abnormal cerebral ultrasound patterns.

**Neurodevelopmental Outcomes**
Neurologic examinations were performed daily, and neonatal neurologic conditions were classified as described by Prechtl (22), with each infant assigned to one of three diagnostic groups: normal, abnormal (when one or more of the following neurologic syndromes were present: hyper- or hypokinesia, hyper- or hypotonia, hemisindrome, apathy syndrome, hyperexcitability syndrome), or suspect (if only isolated symptoms were present but no defined syndrome was evident).

**Laboratory Measurements**
Laboratory values in IUGR infants were recorded at admission to neonatal intensive care units for the standard assessment (i.e., erythrocyte count; glucose, urea, creatinine, hemoglobin, and ion concentrations; hematocrit; venous blood pH; venous CO₂ and O₂ partial pressures; base excess). In controls, clinical and laboratory indexes were recorded at birth.
**S100B Measurements**

Maternal blood was collected from the cubital vein at the time of FVW recording and centrifuged immediately at 900g for 10 min; the resulting sera were stored at −70 °C. Serum S100B concentrations were measured by an immunoluminometric assay (Lia-mat Sangtec 100; AB Sangtec Medical), according to the manufacturer’s instructions. Index tests and the reference standard were blind to the results of the other test. 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 a lack of cross-reactivity with other proteins of the S100 family.

**Statistical Analysis**

Clinical data are reported as the mean (SD), and maternal serum S100B concentrations are reported as the median and 95% confidence interval (95% CI). The results of fetal and neonatal monitoring were compared between groups by the Mann–Whitney U-test and by Kruskal–Wallis one-way ANOVA followed by the Dunn post hoc test when the data did not follow a gaussian distribution. Comparisons between proportions were performed with the Fisher exact test. A P value <0.05 was considered significant.

To analyze the influence of various clinical variables [delivery mode, gestational age and weight at birth, tocolytic therapy, antenatal maternal glucocorticoid administration, chorioamnionitis, incidence of respiratory distress syndrome (RDS), mechanical ventilation support, persistence of patent ductus arteriosus, neurologic examination, and S100B blood concentrations] on the occurrence of IVH, we used multiple forward stepwise regression analysis with the occurrence of IVH as the dependent variable. The cutoff points for defining “high” S100B concentrations for prediction of IVH were chosen by ROC curve analysis (23). We used the best cutoffs indicated by the ROC analysis to calculate specificity, sensitivity, positive and negative predictive values with their respective 95% CIs, likelihood ratios, and areas under the curves (24).

**Results**

The characteristics of the studied groups are shown in Tables 1 and 2. As expected, weight and gestational age at birth were significantly (P <0.001) lower in the IUGR groups than in the controls, as were the Apgar scores at 1 and 5 min (P <0.001). Maternal age and racial or ethnic characteristics, gestational age at sampling, inborn/outborn incidence, and sex did not differ significantly among groups (P >0.05 for all); however, the prevalences of antenatal maternal glucocorticoid treatment, gestational hypertension, preeclampsia, cesarean section, chorioamnionitis, and early-onset sepsis were significantly (P <0.05) higher in the IUGR groups than the control group.

**Fetal-Placental Doppler Findings**

The fetal-placental Doppler findings in the control group were appropriate for gestational age. In the IUGR group, however, the prevalence of abnormal fetal-placental FVW patterns was significantly greater (P <0.001 for all): 64 of 106 fetuses (60%) had a significant increase in fetal UA PI

<p>| Table 1. Baseline characteristics for pregnant women studied and monitoring results. |</p>
<table>
<thead>
<tr>
<th>Maternal characteristics</th>
<th>Controls</th>
<th>All IUGR groups</th>
<th>P&lt;sup&gt;a&lt;/sup&gt;</th>
<th>IUGR group</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>P&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. in group</td>
<td>212</td>
<td>106</td>
<td></td>
<td></td>
<td>46</td>
<td>34</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>Mean (SD) maternal age, years</td>
<td>25.7 (4.1)</td>
<td>25.9 (3.5)</td>
<td>NS&lt;sup&gt;c&lt;/sup&gt;</td>
<td>26.3 (2.9)</td>
<td>25.7 (3.9)</td>
<td>25.9 (3.6)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Mother’s racial or ethnic group, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>15 (7.1)</td>
<td>8 (7.5)</td>
<td>NS</td>
<td>4 (8.7)</td>
<td>2 (5.9)</td>
<td>2 (7.7)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>178 (84.0)</td>
<td>89 (84)</td>
<td>NS</td>
<td>38 (82.6)</td>
<td>29 (85.3)</td>
<td>22 (84.6)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>19 (8.9)</td>
<td>9 (8.5)</td>
<td>NS</td>
<td>4 (8.7)</td>
<td>3 (8.8)</td>
<td>2 (7.7)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>UA PI &gt;95th centile, n (%)</td>
<td>0 (0)</td>
<td>64 (60.4)</td>
<td>&lt;0.001</td>
<td>4 (8.7)</td>
<td>34 (100)</td>
<td>26 (100)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>MCA PI &lt;5th centile, n (%)</td>
<td>0 (0)</td>
<td>60 (56.6)</td>
<td>&lt;0.001</td>
<td>0 (0.0)</td>
<td>34 (100)</td>
<td>26 (100)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>UA PI/MCA PI &gt;1, n (%)</td>
<td>0 (0)</td>
<td>60 (56.6)</td>
<td>&lt;0.001</td>
<td>0 (0.0)</td>
<td>34 (100)</td>
<td>26 (100)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Prenatal glucocorticoids, n (%)</td>
<td>11 (5)</td>
<td>52 (49.1)</td>
<td>&lt;0.001</td>
<td>22 (47.8)</td>
<td>17 (50.0)</td>
<td>13 (50.0)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Chorioamniositis, n (%)</td>
<td>13 (6.1)</td>
<td>21 (19.8)</td>
<td>&lt;0.05</td>
<td>9 (19.6)</td>
<td>7 (20.6)</td>
<td>5 (19.2)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Gestational hypertension, n (%)</td>
<td>11 (5.2)</td>
<td>22 (20.7)</td>
<td>&lt;0.05</td>
<td>10 (21.7)</td>
<td>6 (17.6)</td>
<td>6 (23.1)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Preeclampsia, n (%)</td>
<td>7 (3.3)</td>
<td>28 (26.4)</td>
<td>&lt;0.001</td>
<td>12 (26.1)</td>
<td>9 (26.5)</td>
<td>7 (26.9)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Mean (SD) gestational age at sampling, weeks</td>
<td>33.1 (3)</td>
<td>33.0 (3.1)</td>
<td>NS</td>
<td>33.3 (2.5)</td>
<td>33.2 (2.4)</td>
<td>32.6 (3.1)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Maternal blood S100B, μg/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>0.06</td>
<td>0.10</td>
<td>&lt;0.001</td>
<td>0.04</td>
<td>0.12</td>
<td>1.11</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>95% CI</td>
<td>0.05–0.07</td>
<td>0.07–0.17</td>
<td></td>
<td>0.03–0.06</td>
<td>0.07–0.39</td>
<td>0.92–1.35</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> P for differences between controls vs total IUGR group.

<sup>b</sup> P for differences among IUGR group.

<sup>c</sup> NS, not significant.
and 60 (57%) had a significant decrease in the MCA PI (below 5th centile). Only 60 IUGR fetuses had a UA PI/MCA PI ratio (brain-sparing effect). The fetal-placental FVW patterns in the remaining 46 IUGR fetuses were within the range of normality, not different from those in the controls ($P > 0.05$, for all; Table 1).

### NEONATAL OUTCOMES

The prevalences of RDS, patent ductus arteriosus, pneumothorax, necrotizing enterocolitis, and the need of mechanical ventilation were significantly ($P < 0.001$ for all) higher in the IUGR group than in the control group (Table 2). None of the infants in the control group showed neurologic abnormalities at the time of discharge from the hospital, and no overt neurologic syndromes were observed during recovery. Cerebral ultrasound patterns recorded within the first 72 h after delivery and at the time of discharge from the hospital were negative for CNS malformations, cerebral bleeding, and periventricular leukomalacia.

In the IUGR group, neurologic examination at admission to the neonatal intensive care units showed 22 cases with isolated and transient symptoms, including hyper-/hypotonia ($n = 14$), dystonia ($n = 4$), and hyperexcitability ($n = 4$). Abnormal neurologic patterns were observed in 18 IUGR infants, including hyper-/hypotonia ($n = 8$), dystonia ($n = 6$), and hyperexcitability ($n = 4$), whereas neurologic patterns were normal in 66 IUGR newborns (Table 2). Four of 106 IUGR infants died during the first week after birth from cardiopulmonary failure ($n = 2$) or cerebral bleeding complications ($n = 2$).

Cerebral ultrasound examinations performed within 72 h after birth showed IVH in 26 of 106 IUGR newborns (Table 2), and in all of these cases the brain-sparing effect was observed prenatally (Table 1). As expected, the per-

### Table 2. Baseline of neonatal outcomes.

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>All IUGR groups</th>
<th>$P^a$</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>$P^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. in group</td>
<td>212</td>
<td>106</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD) gestational age at delivery, weeks</td>
<td>38.1 (3)</td>
<td>33.0 (2.6)</td>
<td>&lt;0.001</td>
<td>33.5 (2.5)</td>
<td>32.5 (3.1)</td>
<td>32.6 (3.1)</td>
<td>NS$^c$</td>
</tr>
<tr>
<td>Mode of delivery, n (%)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>Caesarian</td>
<td>48 (22.6)</td>
<td>90 (84.9)</td>
<td>&lt;0.001</td>
<td>39 (84.8)</td>
<td>29 (85.3)</td>
<td>22 (84.6)</td>
<td>NS</td>
</tr>
<tr>
<td>Vaginal</td>
<td>164 (77.4)</td>
<td>16 (15.1)</td>
<td>&lt;0.001</td>
<td>7 (15.2)</td>
<td>5 (14.7)</td>
<td>4 (15.4)</td>
<td>NS</td>
</tr>
<tr>
<td>Birth weight, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;10th centile</td>
<td>0 (0)</td>
<td>106 (100)</td>
<td>&lt;0.001</td>
<td>46 (100)</td>
<td>34 (100)</td>
<td>26 (100)</td>
<td>NS</td>
</tr>
<tr>
<td>10th–90th centiles</td>
<td>212 (100)</td>
<td>0 (0)</td>
<td>&lt;0.001</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>NS</td>
</tr>
<tr>
<td>Born at study hospitals, n (%)</td>
<td>144 (67.9)</td>
<td>70 (66)</td>
<td>NS</td>
<td>31 (67.4)</td>
<td>22 (64.7)</td>
<td>17 (65.4)</td>
<td>NS</td>
</tr>
<tr>
<td>Apgar score &lt;7, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At 1 min</td>
<td>0 (0)</td>
<td>24 (22.6)</td>
<td>&lt;0.001</td>
<td>10 (21.7)</td>
<td>8 (23.5)</td>
<td>6 (23.1)</td>
<td>NS</td>
</tr>
<tr>
<td>At 5 min</td>
<td>0 (0)</td>
<td>13 (12.3)</td>
<td>&lt;0.001</td>
<td>6 (13.0)</td>
<td>4 (11.8)</td>
<td>3 (11.5)</td>
<td>NS</td>
</tr>
<tr>
<td>Sex, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>119 (56.1)</td>
<td>56 (52.8)</td>
<td>NS</td>
<td>24 (52.2)</td>
<td>18 (52.9)</td>
<td>14 (53.8)</td>
<td>NS</td>
</tr>
<tr>
<td>Female</td>
<td>93 (43.9)</td>
<td>50 (47.2)</td>
<td>NS</td>
<td>22 (47.2)</td>
<td>16 (47.1)</td>
<td>12 (46.2)</td>
<td>NS</td>
</tr>
<tr>
<td>Neonatal outcome, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>RDS</td>
<td>27 (12.7)</td>
<td>32 (30.2)</td>
<td>&lt;0.001</td>
<td>14 (30.4)</td>
<td>10 (29.4)</td>
<td>8 (30.8)</td>
<td>NS</td>
</tr>
<tr>
<td>PDA</td>
<td>41 (19.3)</td>
<td>37 (34.9)</td>
<td>&lt;0.001</td>
<td>16 (34.8)</td>
<td>12 (35.3)</td>
<td>9 (34.6)</td>
<td>NS</td>
</tr>
<tr>
<td>Necrotizing enterocolitis</td>
<td>0 (0)</td>
<td>2 (1.9)</td>
<td>NS</td>
<td>0 (0)</td>
<td>1 (2.9)</td>
<td>1 (3.8)</td>
<td>NS</td>
</tr>
<tr>
<td>Pneumothorax</td>
<td>1 (0.5)</td>
<td>2 (1.9)</td>
<td>NS</td>
<td>0 (0)</td>
<td>1 (2.9)</td>
<td>1 (3.8)</td>
<td>NS</td>
</tr>
<tr>
<td>Early-onset sepsis</td>
<td>5 (2.4)</td>
<td>18 (17.0)</td>
<td>&lt;0.05</td>
<td>8 (17.4)</td>
<td>6 (17.6)</td>
<td>4 (15.4)</td>
<td>NS</td>
</tr>
<tr>
<td>Prechtl score at admission to NICU</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>212 (100)</td>
<td>66 (62.3)</td>
<td>&lt;0.001</td>
<td>29 (63.0)</td>
<td>21 (61.8)</td>
<td>16 (61.5)</td>
<td>NS</td>
</tr>
<tr>
<td>Suspect</td>
<td>0 (0)</td>
<td>22 (20.7)</td>
<td>&lt;0.001</td>
<td>10 (21.7)</td>
<td>7 (20.6)</td>
<td>5 (19.2)</td>
<td>NS</td>
</tr>
<tr>
<td>Abnormal</td>
<td>0 (0)</td>
<td>18 (17.0)</td>
<td>&lt;0.001</td>
<td>8 (17.4)</td>
<td>6 (17.6)</td>
<td>4 (15.4)</td>
<td>NS</td>
</tr>
<tr>
<td>Survival, n (%)</td>
<td>212 (100)</td>
<td>102 (96.2)</td>
<td>&lt;0.001</td>
<td>1 (2.2)</td>
<td>1 (2.9)</td>
<td>2 (7.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IVH, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>26 (24.5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 1</td>
<td>2 (1.9)</td>
<td>0 (0)</td>
<td></td>
<td>0 (0)</td>
<td>2 (7.7)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Grade 2</td>
<td>8 (7.5)</td>
<td>0 (0)</td>
<td></td>
<td>0 (0)</td>
<td>8 (30.8)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Grade 3</td>
<td>9 (8.5)</td>
<td>0 (0)</td>
<td></td>
<td>0 (0)</td>
<td>9 (34.6)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Grade 4</td>
<td>7 (6.6)</td>
<td>0 (0)</td>
<td></td>
<td>0 (0)</td>
<td>7 (26.9)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ $P$ for difference between controls and total IUGR group.

$^b$ $P$ for difference among IUGR groups.

$^c$ NS, not significant; PDA, patent ductus arteriosus; NICU, neonatal intensive care unit.
controls and group A; 100%) and a specificity of 99.3% (95% CI, 97.5%–99.9%) as S100B concentrations for prediction of IVH were chosen.  

We evaluated according to the occurrence of IVH and the presence of the brain-sparing effect, we found that group C (n = 26; with brain-sparing effect and later developing IVH) had the highest maternal S100B concentrations, significantly (P <0.001) higher than in the controls, group A (n = 46; without brain-sparing effect and with normal cerebral ultrasound patterns), or group B (n = 34, with brain-sparing effect, normal cerebral ultrasound patterns, but not developing IVH; Fig. 1). Maternal S100B concentrations in both groups B and C were significantly (P <0.001 for both) higher than in group A or controls, whereas they did not differ significantly (P >0.05) between group A and the controls (Fig. 1). Multiple forward stepwise regression analysis with the occurrence of IVH as the dependent variable, performed to assess the influence of various clinical variables on the occurrence of IVH, showed a positive significant correlation (r = 0.71; P <0.001) with maternal S100B concentrations.

The cutoff points for defining high maternal serum S100B concentrations for prediction of IVH were chosen by ROC curve analysis. S100B at the cutoff value of 0.72 µg/L achieved a sensitivity of 100% (95% CI, 86.7%–100%) and a specificity of 99.3% (95% CI, 97.5%–99.9%) as a single marker for predicting IVH in IUGR newborns (area under the ROC curve, 0.999), with positive and negative likelihood ratios of 146 and 0, respectively. Twenty-six of 318 newborns developed IVH, giving an overall prevalence of the disease in the study population of 8.2%. This was the pretest probability (predicted probability of a newborn developing IVH before maternal S100B was measured and the brain-sparing effect was computed). With respect to the early prediction of IVH based on presence of brain sparing alone, if the brain-sparing effect was detected, the probability of a newborn developing IVH (positive predictive value) was 43.0% (95% CI, 30.5%–55.5%), whereas if no brain-sparing effect was detected, the probability of developing IVH (100% − negative predictive value) was 100% (95% CI, 98.0%–100%). When measured maternal S100B concentrations were high (i.e., above the threshold defined by the ROC curve analysis), the probability of an IUGR newborn developing IVH was as high as 93% (95% CI, 83.6%–100%), whereas with maternal S100B values below the cutoff, the probability was 0% (95% CI, 0%–2.5%; Table 3). When both the brain-sparing effect was present and maternal S100B concentrations were high, the probability of an infant developing IVH was 93% (95% CI, 83.6%–100%), whereas when either the brain-sparing effect was absent or maternal S100B values were below the cutoff, the negative predictive value was 0% (95% CI, 0%–2.5%), similar to that obtained by use of the S100B measurement alone (Table 3).

**MATERNAL SERUM S100B CONCENTRATIONS**

S100B was detectable in all maternal serum samples. In controls, S100B concentrations did not change with advancing gestational age. S100B concentrations were significantly (P <0.001) higher in pregnancies complicated by IUGR than in control pregnancies (Table 1). When evaluated according to the occurrence of IVH and the presence of the brain-sparing effect, we found that group C (n = 26; with brain-sparing effect and later developing IVH) had the highest maternal S100B concentrations, significantly (P <0.001) higher than in the controls, group A (n = 46; without brain-sparing effect and with normal cerebral ultrasound patterns), or group B (n = 34, with brain-sparing effect, normal cerebral ultrasound patterns, but not developing IVH; Fig. 1). Maternal S100B concentrations in both groups B and C were significantly (P <0.001 for both) higher than in group A or controls, whereas they did not differ significantly (P >0.05) between group A and the controls (Fig. 1). Multiple forward stepwise regression analysis with the occurrence of IVH as the dependent variable, performed to assess the influence of various clinical variables on the occurrence of IVH, showed a positive significant correlation (r = 0.71; P <0.001) with maternal S100B concentrations.

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**Discussion**

It is commonly accepted that IUGR is associated with utero-placental blood flow impairment, which may be reduced up to 50% (25), so that transfer of oxygen and nutrients from the mother to the fetus is diminished, leading to a cardiovascular response characterized by a redistribution of cardiac output to maintain oxygen supply to the brain, heart, and adrenals to preserve their function at the expense of visceral organs (26). This redistribution phenomenon, called the brain-sparing effect, correlates with the degree of fetal hypoxia and the perinatal outcome (27–29); adverse effects of hypoxemia on brain maturation have been demonstrated in clinical and histologic studies (30–32). In the present study we found that maternal serum S100B concentrations were high in pregnancies complicated by IUGR with blood flow redistribution. These data and the findings that, in cord blood from pregnancies complicated by IUGR, S100B concentrations negatively correlated with a decrease in the Doppler PI in the MCA (33) strongly support an association between increases in S100B concentrations and redistribution of blood flow toward the cerebral circulation during fetal hypoxia.

The highest S100B concentrations were observed in women carrying an IUGR fetus who developed IVH after birth. Previous evidence that (a) S100B is present in higher concentrations in the fetal than in maternal circulation.
Brain sparing, S100B concentrations normal 0 258 258 100 (98–100)

Both altered 26 2 28 93 (83.6–100)

Both normal 0 258 258 100 (98–100)

High S100B concentration, no brain sparing 0 0 0 100 (98–100)

Brain sparing, S100B concentrations normal 0 258 258 100 (98–100)

* Values in parentheses are the 95% CI, calculated as in Ref. (25).

b PPV, positive predictive value; NPV, negative predictive value.

(33), (b) the major source of S100B is the CNS (4), and (c) S100B concentrations are increased in several biological fluids in the presence of brain injury (6, 7, 9–16) suggest that increased S100B concentrations in those mothers may reflect fetal cellular brain damage attributable to chronic hypoxia. One possibility is that the permeability of the blood–brain barrier changes as a result of fetal hypoxia. Alternatively, increased cerebral perfusion secondary to redistribution of blood flow may promote leakage of the protein from the CNS into the fetal circulation (29, 34), as shown previously (29).

The possibility that S100B may be released, at least in part, from the placenta during hypoxic conditions could also be considered. S100B is localized in villous and intermediate trophoblast cells; however, its expression does not change between uncomplicated and IUGR pregnancies (35). In any case, one should assume that large increases in S100B concentrations in fetal and/or placental tissues would lead to an appreciable increase in the maternal blood in spite of the dilution effect attributable to the higher volume of the maternal bloodstream. Obviously, S100B can also derive from maternal tissues, as reported recently (36). Because all pregnant women enrolled in the present study were in healthy condition and had no detectable overt neurologic syndromes, the possibility that the protein originates from damaged maternal nervous system cells seems fairly remote.

The significance of such increased S100B concentrations warrants consideration. At nanomolar concentrations, S100B stimulates neurite outgrowth and enhances survival of neurons during development (37), whereas micromolar concentrations of extracellular S100B in vitro stimulate the expression of proinflammatory cytokines and induce apoptosis (38, 39). On the other hand, cell-based and clinical studies have implicated S100B in the initiation and maintenance of a pathologic, giall-mediated proinflammatory state in the CNS (40). Overexpression of S100B increases vulnerability to cerebral hypoxic-ischemic injury; for example, in a study of S100B transgenic mice subjected to hypoxia-ischemia, the incidence of mortality increased significantly as a result of more extensive cerebral injury and neuroinflammation in response to injury (40). The possibility that some of the S100B derives from this process and participates in the pathologic cascade of events responsible for IVH should also be taken into account.

In the present study we also found that measuring S100B in mothers carrying an IUGR fetus before there is any ultrasound pattern suggestive of CNS damage, and at a stage when standard diagnostic procedures are still silent or not applicable, yields positive and negative predictive values that differ materially from the overall prevalence of IVH in the study population. This indicates that the probability of a newborn developing IVH may be estimated more precisely if maternal S100B measurements are performed after detection of the brain-sparing effect. It thus follows that S100B cannot be increased solely during conditions of cerebral ischemia, and hence cerebral damage, but it is better related to fetal hypoxic stress, which leads to redistribution of blood flow by triggering a chemoreflex response. As such, rather than as a marker of fetal brain damage per se, detection of increases in S100B may better serve as an early clinical marker of fetal hypoxia, particularly in combination with evidence of redistribution of blood flow, which if persistent may lead to fetal brain damage.

We found that mothers with serum S100B concentrations above the threshold defined by the ROC curve analysis (>0.72 μg/L) had a probability (positive predictive value) of delivering a newborn who later would develop IVH as high as 93% (with a positive likelihood ratio of 62.0), whereas the probability was 0% if S100B concentrations were not above the threshold, sharing positive and negative predictive values that differed from the overall IVH prevalence (8.2%) in the study population.
Our findings indicate that biochemical markers in the maternal bloodstream may be useful for the early detection of fetuses at higher risk of postnatal neurologic sequelae and may possibly be used to prevent unnecessary interventions. In our example, S100B measurements were used to improve the ability to predict IVH in IUGR fetuses diagnosed on the basis of abnormal Doppler velocimetry, which was used as a first step screening test for the general population, at a time when clinical, laboratory, and ultrasound patterns were unable to detect cases at risk of IVH.

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References


