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S100B maternal blood levels are gestational age- and gender-dependent in healthy pregnancies

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Abstract

Background: S100B is a well-established biomarker of central nervous system (CNS) development and damage in the perinatal period. Because the fetal CNS induces an overproduction of S100B measurable in the maternal bloodstream we evaluated S100B protein in healthy pregnancies in order to provide a reference curve of the protein in the second and third trimesters and to provide information on CNS development when standard monitoring procedures could be silent or unavailable.
Methods: Between July 2012 and December 2014 we conducted a prospective study in 1213 healthy pregnancies delivering healthy newborns. Maternal blood samples were collected for standard monitoring procedures and S100B assessment. S100B correlations with selected outcomes (gestational age at sampling, gender of fetus, gestational age and weight at birth, delivery mode) were calculated using multiple forward stepwise regression analysis.
Results: S100B concentrations in the second and third trimesters of pregnancy were found to be gestational age-, gender- and delivery mode-dependent ($p < 0.05$, for all). Multiple forward stepwise regression analysis with S100B as the dependent variable and gestational age at sampling, gender, delivery mode, gestational age and weight at birth as independent variables, showed a significant correlation between S100B and gestational age at sampling ($R = 0.13$; $p < 0.001$).
Conclusions: The present findings offering a S100B protein reference curve in maternal blood suggest that non-invasive fetal CNS monitoring is becoming feasible and open the way to further research in neuro-biomarker assessment in the maternal bloodstream.

Keywords: biomarker; brain development; fetus; newborn; pregnancy; S100B.

Introduction

There is growing evidence that the vast majority of neurological abnormalities present during childhood are due to pre-perinatal adverse events [1]. Thus, information concerning the timing of insult is of great importance with regard to future preventive measures [2]. The timing of the insult and of contributing factors may be improved by adequate documentation of general medical and obstetrical factors and by standard laboratory and ultrasound monitoring procedures [3]. Despite considerable progress, central nervous system (CNS) development/damage monitoring throughout pregnancy still constitutes a challenge for perinatologists. This is especially true for new diagnostic and monitoring tools to be included in daily clinical practice. In this regard, it has recently been suggested that the assessment of brain biomarkers [4–6] in biological fluids can offer useful information on CNS development and injury at a time when standard monitoring procedures may still be silent or unavailable [7].

In the panel of biomarkers currently investigated for perinatal CNS monitoring, S100B is of particular interest due to its dual role: trophic factor at nanomolar concentrations and brain damage marker at micromolar concentrations [8]. S100B is an acidic calcium-binding brain specific protein mainly concentrated in the glial cells, astrocytes, Schwann cells, and in neurons [4–6]. The protein has been reported to be involved in the regulation

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of several cellular functions (cell-cell communication, cell growth, cell structure, energy metabolism, contraction, and intracellular signal transduction) and in the cascade of events leading to neuronal death and apoptosis [5]. In different biological fluids (i.e. cerebrospinal fluid, blood, urine, amniotic fluid and saliva), S100B has been shown to be a reliable marker of CNS development and injury [9–19]. More recently, elevated maternal blood protein concentrations have been detected in pregnancies complicated by intrauterine growth restriction and postnatal intraventricular hemorrhage [20], whilst in healthy pregnancies the presence of a protein gradient between maternal and fetal bloodstreams has been shown [21]. Although some results suggest that fetal CNS monitoring is becoming feasible, a S100B protein reference curve for maternal blood during the second and third trimesters of pregnancy is still lacking.

In the present study we therefore investigated the pattern of S100B concentration in the second and third trimesters in a cohort of 1213 pregnant women, in order to provide a reference curve of the protein in the period under investigation and its correlations with gestational age, gender and delivery mode.

Materials and methods

Informed consent was obtained from all women before inclusion in the study, and approval was obtained from our Local Human Investigation Committees.

Based on our epidemiological data (about 5.000 deliveries per year, including the three hospitals referring to our III level for obstetrics and neonatal care), we conducted a prospective study from July 2012 to December 2014. The sample size calculation estimated the recruitment of a cohort of 80 assessments for every 2-weeks of gestation (WG) during the second (13–26 WG) and third (27–40 WG) trimesters: we therefore recruited 1213 pregnant women. According to Leslie and Greenberg [22] that a cohort of less than 50 cases causes high imprecision, we found that a cohort of 80 cases every 2-WG reached a precision of 0.5 referred to a confidence interval of 95%.

Healthy pregnancies and newborns were defined according to the criteria of the American College of Obstetrics and Gynecology and the American Academy of Pediatrics. In detail, gestational age was determined by the last menstrual period and confirmed by a first trimester ultrasound scan. Appropriate growth was defined by the presence of ultrasonographic signs (biparietal diameter and abdominal circumference between the 10th and 90th centiles), according to the normograms of Campbell and Thoms [23], and by post-natal confirmation of a birth weight between the 10th and 90th centiles according to our population standards, correcting for the mother's height, weight, parity and the sex of the newborn. At birth, newborns fulfilling all of the following criteria were classified as normal: 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.

Exclusion criteria were: multiple pregnancies, gestational diabetes or any maternal CNS illness, and pregnancies carrying a fetus with any malformation, systemic infection, intrauterine growth retardation, or cardiac or hemolytic disease.

In all women, according to national guidelines, maternal blood was drawn from the cubital vein for standard laboratory investigations and S100B protein assessment at six pre-determined monitoring time-points (T1: 13–18 WG; T2: 19–23 WG; T3: 24–28 WG; T4: 29–32; T5: 33–37 WG; T6: > 37 WG).

S100B measurement

Maternal blood samples collected at different WG were centrifuged at 3500 *g* for 10' and supernatant stored at – 70 °C until assessment. The S100B protein concentration was measured in all samples using a commercially available immunoluminometric assay (Liaison S100, Dietzenbach, Germany). The limit of detection of the assay was 0.02 µg/L. The precision was 6.74% or lower within-assay and 9.34% or lower inter-assay calculated by five replies in 15 different days for a S100B concentration at 0.04 µg/L.

Statistical analysis

S100B concentrations in maternal blood were expressed as the median and 25th–75th centiles. Data were analyzed for statistically significant differences between groups by the Mann-Whitney U two-sided test when not normally distributed. The correlation between S100B maternal blood concentrations and age at sampling was assessed by linear regression analysis and by a polynomial regression analysis type-2. To analyze the influence of various clinical variables (gestational age at sampling, delivery mode, gestational age and weight at birth, gender) on S100B blood concentrations we used multiple forward stepwise regression analysis with S100B as the dependent variable. Statistical significance was set at $p < 0.05$.

Results

Table 1 shows the demographic characteristics and main perinatal outcomes. As expected, all pregnant women delivered healthy newborns showing good clinical condition and no overt neurological diseases on discharge from the hospital.

Maternal blood S100B protein was detectable in all samples evaluated. The pattern of concentration of the protein in the second and third trimesters of pregnancy showed a progressive decrease from the 12th to the 29th WG, followed by an increase from the 30th to the 36th WG and, finally, by a drop in the protein near term. The median and interquartile ranges for S100B at different WG are reported in Table 2.

Table 1: Demographic, perinatal clinical characteristics and outcomes of the studied group.

Parameters	Healthy pregnancies and newborns (n = 1213)
Maternal age, years	25.6 ± 4.4
Delivery mode, n (%)	
Cesarean	250 (20)
Vaginal	963 (80)
Gestational age (weeks)	40 ± 1
Birth weight, n (%)	
< 10th centile	0 (0)
10th–90th centiles	1213 (100)
Apgar score > 7, n (%)	
At 1 min	1213 (100)
At 5 min	1213 (100)
Gender (male/female)	631/582
Ethnicity, n (%)	
Causasian	1042
Black	35
Asiatic	18
South American	118
Blood pH > 7.20, n (%)	1213 (100)
Neurological examination at admission, n (%)	
Normal	1213 (100)
Suspect	0 (0)
Abnormal	0 (0)

Values are expressed as mean ± SD and percentages.

S100B measured in the second trimester of pregnancy was significantly higher ($p < 0.001$) at 13–14 WG (median, 0.05 μg/L; 25th–75th percentiles, 0.04–0.07 μg/L) than that detected at 21–22 WG (median, 0.04 μg/L; 25th–75th percentiles, 0.02–0.05 μg/L). No S100B differences were observed at other second trimester WG ($p < 0.05$, for all).

S100B measured in the third trimester at 27–28 WG was significantly higher ($p < 0.05$) (median, 0.04 μg/L; 25th–75th percentiles, 0.02–0.07 μg/L) than at 31–32 WG (median, 0.03 μg/L; 25th–75th percentiles, 0.02–0.05 μg/L), and was lower ($p < 0.05$) than that at term WG (median, 0.05 μg/L; 25th–75th percentiles, 0.04–0.08 μg/L). Moreover, no S100B differences were detected at other WG ($p < 0.05$, for all).

The pattern of S100B concentration at different WG after correction for fetal gender is shown in Figure 1. No differences were found when we compared total male and female S100B in the second and third trimester WG ($p > 0.05$, for both). In the second trimester of pregnancy, S100B was higher ($p < 0.05$) in male fetuses at 25–26 WG (median, 0.05 μg/L; 25th–75th percentiles, 0.03–0.07 μg/L) than in female fetuses (median, 0.04 μg/L; 25th–75th percentiles, 0.02–0.06 μg/L). No differences in S100B were observed at other WG ($p < 0.05$, for all).

Table 2: S100B maternal blood levels (μg/L) measured at different weeks' gestation (GA) of the II–III trimesters of healthy pregnant women who delivered healthy newborns.

	Studied group (n = 1213)		
	Median	25° centile	75° centile
II Trimester (n = 562)			
13–14	0.05	0.04	0.07
15–16	0.04	0.03	0.07
17–18	0.04	0.03	0.06
19–20	0.04	0.03	0.07
21–22	0.04	0.02	0.05
23–24	0.05	0.03	0.07
25–26	0.04	0.03	0.07
III Trimester (n = 651)			
27–28	0.04	0.02	0.07
29–30	0.04	0.03	0.06
31–32	0.03	0.02	0.05
33–34	0.05	0.03	0.07
35–36	0.04	0.03	0.06
> 37	0.05	0.04	0.08

Data are shown as median and interquartile ranges.

In the third trimester S100B measured at 35–36 WG was significantly higher ($p < 0.01$) in female (median, 0.05 μg/L; 25th–75th percentiles, 0.04–0.07 μg/L) than in male fetuses (median, 0.04 μg/L; 25th–75th percentiles, 0.02–0.06 μg/L); at term, S100B was higher ($p < 0.05$) in female (median, 0.06 μg/L; 25th–75th percentiles, 0.05–0.10 μg/L) than in male fetuses (median, 0.05 μg/L;

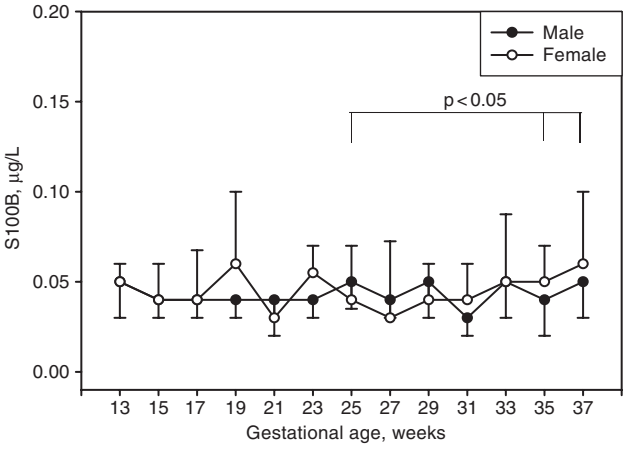

Figure 1: S100B maternal blood levels (μg/L) measured at II–III trimester of gestations after correction for gender (male ●; female: ○) in healthy pregnant women who delivered healthy newborns. Data are shown as median and interquartile ranges. S100B levels were significantly higher in male at 25–26 weeks ($p < 0.05$) whilst from 35 weeks to term protein' levels were significantly higher in female ($p < 0.05$, for both).

Table 3: S100B maternal blood levels (μg/L) measured at different GA weeks of the II–III trimester of healthy pregnant women corrected for delivery mode.

GA, weeks	Vaginal delivery (n = 963)			Cesarean detection (n = 250)			p-Value
	Median	25° centile	75° centile	Median	25°centile	75° centile	
13–14	0.05	0.04	0.07	0.045	0.02	0.07	0.25
15–16	0.04	0.03	0.06	0.04	0.03	0.05	0.52
17–18	0.04	0.03	0.06	0.05	0.02	0.07	0.78
19–20	0.05	0.03	0.07	0.03	0.03	0.06	0.12
21–22	0.04	0.02	0.05	0.03	0.02	0.05	0.31
23–24	0.04	0.03	0.06	0.05	0.03	0.08	0.33
25–26	0.04	0.03	0.07	0.05	0.04	0.08	0.17
27–28	0.04	0.02	0.06	0.05	0.03	0.09	0.20
29–30	0.04	0.02	0.07	0.04	0.03	0.06	0.91
31–32	0.03	0.02	0.05	0.03	0.02	0.06	0.60
33–34	0.05	0.03	0.07	0.05	0.03	0.08	0.40
35–36	0.04	0.03	0.06	0.04	0.03	0.06	0.67
> 37	0.05	0.04	0.08	0.06	0.04	0.09	0.04

Data are shown as median and interquartile ranges.

25th–75th percentiles, 0.03–0.08 μg/L). No S100B differences were observed at other WG ($p < 0.05$, for all).

Table 3 shows S100B at different WG after correction for delivery mode. No significant S100B differences were found when we compared total S100B levels vaginal delivery vs. cesarean section in the second and third trimester WG ($p > 0.05$, for both). Of note, S100B levels measured > 37 WG were significantly higher ($p < 0.05$) in cases delivered by cesarean section (median, 0.05 μg/L; 25th–75th percentiles, 0.03–0.07 μg/L, respectively) than in vaginal deliveries (median, 0.04 μg/L; 25th–75th percentiles, 0.03–0.07 μg/L).

S100B in maternal blood was shown to be correlated with WG at sampling: in the second trimester we found a negative correlation between S100B and WG ($r = -0.15$; $p < 0.001$) while in the third trimester a significant correlation was observed using a polynomial type-2 model ($R = 0.22$; $p < 0.05$).

Multiple forward stepwise regression analysis with S100B as the dependent variable and gestational age at sampling, gender, delivery mode, gestational age and weight at birth as independent variables showed a significant correlation between S100B and gestational age at sampling ($R = 0.13$; $p < 0.001$).

Discussion

Pre-perinatal insults, including preterm birth and intrapartum complications, account for more than 40% of perinatal mortality and, in survivors, 32% of short/long-term morbidity [1, 2]. For these reasons, improvements in the monitoring of the mother and fetus dyad are of immense relevance. In this regard, there is growing evidence that the monitoring of perinatal biomarkers in non-invasive biological fluids is a promising avenue of investigation.

In the present study, we found that levels of a brain-specific protein, S100B, in the maternal bloodstream are gestational age-, gender- and delivery mode-dependent. Multivariable analysis showed that, among a series of perinatal variables, S100B correlated with gestational age at sampling. More importantly, we offer for the first time, a reference curve for S100B protein in the maternal bloodstream in the second and third trimesters of pregnancy.

The presence of fetal S100B in the maternal bloodstream is not surprising and is in agreement with previous results in both healthy and high-risk pregnancies [20, 21]. The pattern of concentration of S100B in the second and third trimesters of gestation warrants further consideration. In particular: (i) in the second trimester, S100B levels were at their highest levels and progressively decreased from the 17th WG onwards, reaching their lowest point at the 21st WG. The pattern of concentration in the 14th–18th WG is in agreement with the trend for this protein reported in amniotic fluid of healthy pregnancies [24]. Data obtained after the 18th WG are not comparable, because of ethical limitations to performing amniocentesis after the period suggested for genetic investigations; (ii) in the third trimester S100B showed a progressive increase in protein levels, peaking at the 36th–37th WG, after which it started to decrease near to term. This pattern is superimposable on that observed in other biological fluids (i.e. cord blood, urine and saliva) of healthy newborns [16–18].

In particular, the peak in the concentration of S100B in maternal blood at 34–36 WG is identical to the pattern previously described in urine [25]. This finding is important, given that late preterm infants account for about 70%–75% of all premature infants [26]. Bearing in mind the absence of any maternal (CNS diseases), pregnancy (diabetes and hypertension, placental insufficiency) or fetal (acute and/or chronic hypoxia) complications known to affect the release of S100B from the CNS, the present findings corroborate the notion of the fetal origin of S100B detected in the maternal bloodstream due to a physiological gradient-mediated transfer from the fetus to the mother [21].

The mechanism through which S100B once released from CNS in the fetal bloodstream, is transported through placenta warrants further consideration. In particular, there is evidence that S100B: (i) is able to pass, under physiological conditions, trough different biological fluids and barriers due to its low molecular weight [4–6], (ii) is transported from systemic blood to urine and from blood to saliva trough a passive mechanisms since no differences in protein' levels among different biological fluids have been shown [16–18], (iii) in adults is a trustable marker of changes in brain blood barrier permeability under physiological and pathological conditions [27], and (iv) levels are significantly higher in fetal than in maternal bloodstream [21]. Altogether, it is reasonable to argue that, as for nutrients and active molecules, placenta plays a regulating crucial role. Further studies, in this setting are so justified. Furthermore, the possibility that the total amount of S100B detected maternal blood could derive from placental source is still controversial and matter of debate. From one side, S100B presence in feto-placental tissues has been shown from the other side no differences in the localization or intensity of protein's staining between uncomplicated and intrauterine growth retarded (IUGR) pregnancies were observed [28, 29]. However, as recently reported in adults, it is reasonable to conclude that placenta constitutes a very low percentage of S100B in extra-cerebral sources compared to circulating protein amount [30].

These findings suggest that fetal CNS monitoring is becoming possible through the most suitable non-invasive biological fluid available, maternal blood. In view of the main dual role of S100B (i.e. neurotrophic factor and marker of hypoxia and brain damage), the present data further support the possibility of early detection of cases complicated by acute/chronic hypoxia and post-natal adverse outcome.

Results in humans and animal models show that fetal development is gender-dependent [31], and perinatal S100B concentrations in biological fluids differ between male and female fetuses and newborns [17, 32]. In particular, S100B levels in cord blood and urine fluids in the third trimester are higher in female than in male fetuses [16, 17]. These data are in agreement with those detected in maternal blood showing higher protein levels in the late preterm and term periods [25]. More interestingly, at the 25th WG higher S100B levels were observed in male fetuses. These findings are corroborated by previous biochemical, morphological and electrophysiological observations of fetal/neonatal CNS maturation, and by ultrasound and MRI patterns [25, 33, 34].

In the present series we also found higher S100B concentrations in maternal blood of pregnancies delivering by cesarean section compared with vaginal delivery. Of note, the difference was statistically significant from the 37th WG onwards (median delivery time: 40 WG), 3 weeks before birth. This finding deserves further consideration. In particular, it has been shown that S100B increases: (i) in both maternal and newborn bloodstreams complicated by prolonged labor and vaginal delivery, reasonably due, in the mother, to the long-lasting oxidative and/or psychogenic stress and, in the newborn, to compressive conditions of the fetus brain during the vaginal delivery [35], (ii) in term newborns complicated by acute perinatal hypoxia and correlated with perinatal blood pH and abnormal CTG on admission to the labor ward [36], (iii) in a sheep-based model for perinatal asphyxia within 15 min from hypoxic insult [37, 38], and (iii) in relation to nitric oxide- and estrogen-mediated mechanisms occurring in the pre-delivery phase [8, 39]. Altogether, since delivery is triggered by physiological hypoxia-, vascular- and hormone-mediated mechanisms, it is reasonable to suggest that an early increase in S100B maternal blood levels (i.e. 72 h before) of newborns delivered by cesarean section can constitute a promising marker for the timing of delivery. This especially holds for pregnancies complicated by maternal diseases (diabetes, hypertension, HELLP syndrome), IUGR, premature rupture of membranes and acute hypoxia in whom a correct timing of the delivery can play a crucial role on newborns' quality of life. In this regard, further investigations on S100B maternal blood pattern of concentration in high risk-pregnancies are needed.

Finally, since the usefulness of S100B has been shown, in perinatal medicine, in evaluating the effectiveness/side-effects on the CNS of in-utero therapeutic strategies, it is reasonable to suggest the adoption of longitudinal assessment of this protein for monitoring fetal well-being in both healthy and high-risk pregnancies [40–43].

In conclusion, the present data offering a reference curve for S100B protein in maternal blood suggest that non-invasive fetal CNS monitoring is becoming feasible

and open the way to further investigation of assessments of neuro-biomarkers in the maternal bloodstream.

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