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Original Article

Elevation of serum S100 protein concentration as a marker of ischemic brain damage in extremely preterm infants

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Abstract

Background: Periventricular leukomalacia (PVL) is serious ischemic brain damage that occurs in extreme preterm infants. It is traditionally diagnosed by cranial echography. The purpose of this study was to investigate the relationship between serum S100 calcium-binding protein B (S100B) concentrations and ischemic brain damage, and to find the cutoff value for the early identification of ischemic brain damage in high-risk preterm infants.

Methods: At the age of 3 days, 7 days, 14 days, and 21 days, and before discharge, 22 extremely premature infants (i.e., gestational age <33 weeks) underwent blood sampling to determine the S100B concentrations and cranial echography examinations. The severity of ischemic brain damage in echographic images was scored on a scale of 0–11, and was recorded as the brain echography index (BEI). If the last BEI value was ≥7, the enrolled infants were grouped in the brain damage group.

Results: Eight infants were assigned to the brain damage group and 14 infants were assigned to the no brain damage group. At each age point of the blood samplings, the serum S100B concentrations were significantly higher in the brain damage group than in the no brain damage group. There was a significantly positive correlation between the serum S100B concentrations and the BEI on the same day ($r = 0.738$, $p < 0.001$) and 7 days later ($r = 0.774$, $p < 0.001$). The receiver operating characteristic curve for the serum S100B concentrations showed that the area under curve was 0.985 ($p < 0.001$). The cutoff value of serum S100B of 1.0 μg/L had a sensitivity of 93.8% and specificity of 90.5% for the diagnosis of ischemic brain damage.

Conclusion: An elevation in the serum S100B concentration is highly associated with ischemic brain damage in extreme preterm infants. Ischemic brain damage in a high-risk preterm infant is strongly suggested if the early serum S100B concentration is > 1.0 μg/L.

Keywords: brain damage; neonate; periventricular leukomalacia; preterm infant; S100B

1. Introduction

Periventricular leukomalacia (PVL) is the most common ischemic brain injury in premature infants born at <32 weeks' gestation. Ischemia occurs in the border zone at the end of arterial vascular distributions in the white matter adjacent to the external angle of the lateral ventricles.[1,2] Detecting PVL is important because a significant percentage of surviving premature infants may develop cerebral palsy. Periventricular

Conflicts of interest: The authors declare no conflicts of interest related to the subject matter or materials discussed in this article.

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leukomalacia is traditionally diagnosed when brain echography detects increased periventricular echo densities or cysts.[3,4] However, the hyperechoic appearance of PVL is difficult to distinguish from the normal periventricular halo, and mild periventricular edema may not result in permanent injury.[5] Only 15% of patients with PVL demonstrate periventricular cysts that appear 2–3 weeks after birth.[6] Therefore, serial cranial echographic image studies and follow up are necessary to make a final diagnosis. Some patients initially have normal brain echographic findings, and later develop clinical and delayed imaging findings of PVL. The process of serial echographic examinations is time–consuming. The images of periventricular echo densities and the appearance of periventricular cystic are sometimes nonspecific or delayed. Therefore, it would be better to find a fast and trustworthy measurement to detect this ischemic brain injury early in sick preterm infants.

Different biological markers have been investigated for the early detection of brain damage in newborn infants.[7,8] S100 calcium-binding protein B (S100B) is an acidic calcium-binding protein; it is primarily concentrated in glial cells of the nervous system and involved in the regulation of several cellular processes.[9] Previous reports have demonstrated that an increased S100B concentration in biological fluids occurs in different kinds of brain injuries such as intraventricular hemorrhage (IVH) in preterm babies, asphyxia, stroke, head injury, cerebral palsy, Down's syndrome, and Alzheimer's disease.[10–16] Thus, it has been examined as a potential biological marker in newborn infants complicated by perinatal asphyxia.[8,11,17,18] However, few studies have been conducted in relation to the use of serum S100B concentrations to help identify cases of ischemic brain damage in preterm infants.[18–21] It is worthy to embark on further investigation to elucidate the role of serum S100B in the diagnosis of ischemic brain damage in high-risk preterm infants.

We hypothesized that the early elevation of serum S100B concentration is associated with ischemic brain damage in preterm infants with abnormal brain echographic findings of PVL. Therefore, the purposes of the present study were to investigate the relationship between serum S100B concentrations and ischemic brain damage, and to find a cutoff value to identify brain damage early in high-risk preterm infants.

2. Methods

2.1. Patients

This study was approved by the Institutional Review Board of Chang Gung Memorial Hospital (Keelung, Taiwan; IRB no., 100-3373C). From December 2011 to December 2012, we enrolled extreme preterm infants with a gestational age of <33 weeks into this study, after obtaining their parents' consent. The exclusion criteria were (1) patient death before 28 days old, (2) evidence of IVH of Grade II or higher, (3) significant hyperbilirubinemia that required blood exchange transfusion, or (4) any sign of acute bilirubin encephalopathy. In addition, otherwise "well" preterm infants with a gestational age of 33–36 weeks with no requirement of any drug therapy or respiratory support were also enrolled as the near-term reference group, after obtaining their parents' consent.

2.2. Brain echography examination

The brain echographic examinations were performed using a 5 MHz and 7 MHz transducer (iE33 xMATRIX system; Philips Healthcare, Andover, MA, USA). Serial coronal and parasagittal images were obtained and examined through the infants' anterior fontanelles. In the study groups, the enrolled infants received brain echography examinations at the age of 3 days, 7 days, 14 days, and 21 days, and before being discharged from the hospital, when their postconceptional ages would have been 36–40 weeks. In the well near-term reference group, brain echography was performed at 3 days old (Fig. 1).

Increased echogenicity was considered when the echo density of the brain parenchyma was equal to or higher than the choroid plexus in the coronal and the parasagittal planes. Significant evidence of cysts was considered if there was low echogenic and cystic lesions of at least 5-mm diameter in the infant's brain echographs. The diagnosis of PVL was established based on the brain echographic findings. The severity was graded in accordance with the definitions suggested by de Vries et al.[5]

For statistical comparison, we defined severity by using the brain echography index (BEI) score system (Table 1). If the BEI at the age of 28 days or older was $\geq$7, the infants were grouped into the brain damage group; if the final BEI value was <7, they were grouped into the no brain damage group (Fig. 1).

2.3. Analysis of the S100B concentration

Venous blood (1 mL) was drawn four times when the study group infants were age 3 days, 7 days, 14 days, and 21 days. Venous blood was drawn only once at 3 days of age in the near-term reference group. The blood samples were collected in test tubes without a serum separator or an anticoagulant, allowed to clot, and thereafter centrifuged at 900*g* for 10 minutes. The serum was pipetted into 1 mL Eppendorf tubes and stored at −70°C, until later analyzed for the S100B concentration.

The S100B concentration was measured by immunoluminometric assay [S100B (human) ELISA kit; Abnova Corp., Taipei, Taiwan] in accordance with the manufacturer's instructions. Each measurement was also performed in duplicate in accordance with the manufacturer's recommendations. The averages were recorded. Pursuant to the manufacturer's instructions, the sensitivity of the assay (B_0 ± 3SD) was 0.015 μg/L, the intra-assay coefficient of variability was 3.80%, and interassay coefficient of variability was 7.70% for concentrations ranging between 0.05 μg/L and 2 μg/L.

2.4. Statistical analysis

Data were presented as the mean ± the standard deviation (SD) for the continuous data with normal distribution, and as

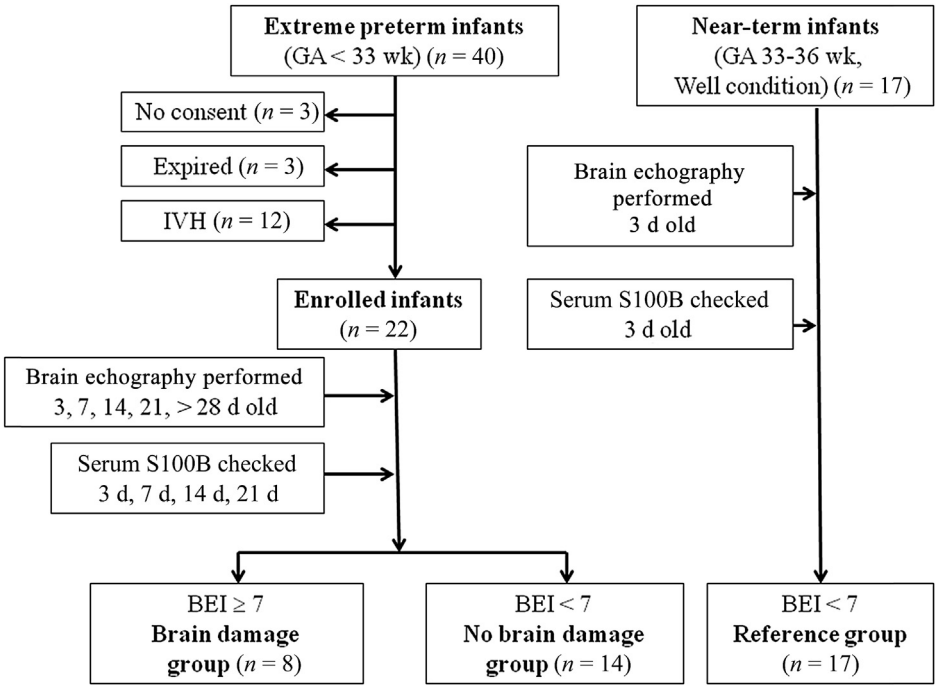

Fig. 1. Flow sheet of patient enrollment and grouping. BEI = brain echography index; GA = gestational age; IVH = intraventricular hemorrhage; S100B = S100 calcium-binding protein B.

the median (range) for the ranked data. Data analysis and graph drawings were performed using SigmaPlot version 12.0 software (Systat Software, Inc.; Point Richmond, CA, USA). We used the independent *t* test or Mann–Whitney *U* test to determine statistical differences between the two groups, when appropriate. One-way repeated measures analysis of variance (ANOVA) was performed to compare data at different age points of the same group. This analysis was then followed by a pairwise comparison with the *post hoc* Bonferroni method. Spearman's correlation coefficient was calculated for analyzing the relationship between serum S100B concentrations and the BEI values. The receiver operating characteristic (ROC) curve of the serum S100B concentrations for predicting brain damage in infants and its area under curve (AUC) analysis were performed using SPSS version 17.0 (SPSS Inc., Chicago, IL, USA). Statistical significance was defined as $p < 0.05$.

Table 1
The brain echography index, based on the severity of periventricular ischemic damage in the infant's brain.

Score[a]	Grade[a]	Echography finding
1	Grade IA	Transient flares with periventricular densities
3	Grade IB	Homogeneous periventricular densities
5	Grade II	Inhomogeneous periventricular densities
7	Grade III	Periventricular densities have evolved into small cysts
9	Grade IV	Periventricular densities have evolved into extensive cysts with ventricular dilatation
11	Grade V	Densities in the periventricular and subcortical regions with extensive and evolving periventricular and subcortical cysts

[a] The scores are modified from the grading system of De Vries et al.[5]

3. Results

A total of 40 extreme preterm infants were born and admitted into our neonatal intensive care unit during the study period. Among them, three infants died, 12 infants developed IVH, and three infants had no parental consent for participation in the study and were therefore excluded from this study. There were ultimately 22 infants enrolled in the study group (Fig. 1). Seventeen well near-term infants were enrolled in the reference group.

The enrolled extreme preterm infants comprised 13 male and nine female infants. They had a mean gestational age of 29.0 ± 2.2 weeks (range, 24–32 weeks) and a mean birth weight of 1246 ± 261 g (range, 728–1572 g). Eight infants were enrolled in the brain damage group, and 14 infants were enrolled in the no brain damage group. A comparison of these two groups revealed that the gestational age, birth weight, Apgar scores at 1 minute and 5 minutes after birth, and blood pH were all significantly higher in the no brain damage group than in the brain damage group (Table 2).

The serum S100B concentrations and the BEI value on the same day had a significantly positive correlation ($r = 0.738$, $p < 0.001$; Fig. 2A). When we compared the S100B concentrations and the BEI values that were obtained 7 days after blood drawing, the correlation coefficient was even higher ($r = 0.774$, $p < 0.001$; Fig. 2B).

A comparison of the S100B concentrations between well extreme preterm infants (i.e., the no brain damage group) and

Table 2
Basic characteristics and summarized laboratory data of the enrolled infants.

Group	Extremely preterm infants			Near-term well infants ($n = 17$)
	All ($n = 22$)	Brain damage ($n = 8$)	No brain damage ($n = 14$)	
Gestational age (wk)	29.0 ± 2.2	27.0 ± 2.1	30.2 ± 1.3*	34.8 ± 0.8
Birth weight (g)	1246 ± 261	1026 ± 252	1372 ± 170*	2469 ± 92
Apgar score at 1 min	6 (1–9)	5 (1–7)	8 (4–9)*	8 (7–9)
Apgar score at 5 min	8 (4–10)**	7 (4–8)	9 (6–10)*	9 (7–10)
Initial laboratory data				
pH	7.31 ± 0.16	7.20 ± 0.16	7.38 ± 0.12*	NA
pCO_2	44 ± 12	50 ± 14	42 ± 10	NA
pO_2	91 ± 28	81 ± 36	96 ± 22	NA
Hemoglobin	16 ± 3	15 ± 3	16 ± 2	NA
Hematocrit	49 ± 10	46 ± 9	51 ± 10	NA
Serum S100 B concentrations				
3 days old	1.00 ± 0.61	1.53 ± 0.69*	0.70 ± 0.28	0.55 ± 0.09**
7 days old	1.57 ± 1.06	2.68 ± 0.90[a,*]	0.93 ± 0.44	NA
14 days old	1.82 ± 1.70	3.79 ± 1.24[a,b,*]	0.70 ± 0.29	NA
21 days old	1.49 ± 1.50	3.18 ± 1.26[a,c,*]	0.52 ± 0.20	NA
Brain echography index				
3 days old	3 (1–3)	3 (1–3)	3 (1–3)	0
7 days old	3 (1–5)	3 (3–5)	3 (1–3)*	NA
14 days old	3 (1–7)	3 (3–7)	1 (1–3)*	NA
21 days old	3 (0–11)	7 (1–11)	1 (0–3)*	NA
>28 days old	4 (0–11)	9 (9–11)	0 (0–1)*	NA

Data are presented as the mean ± standard deviation or median (range), as appropriate.
Between-group comparison: *$p < 0.05$ compared to no brain damage group at the same age and **$p < 0.05$ compared to no brain damage group in extremely preterm infants.
Within group comparison: [a] $p < 0.05$ compared to 3 days old, [b] $p < 0.05$ compared to 7 days old, and [c] $p < 0.05$ compared to 14 days old.
NA = not available; pCO_2 = partial pressure of carbon dioxide; pO2 = partial pressure of oxygen; S100 B = S100 calcium-binding protein B.

the reference near-term infants showed that the S100B concentrations at 3 days of age were significantly higher in the extreme preterm infants than in the near-term infants ($p < 0.05$; Table 2).

A comparison of the between-group serum S100B concentrations in the enrolled extreme preterm infants showed that the concentrations were significantly higher in the brain damage group than in the no brain damage group at each age point ($p < 0.05$; Fig. 3). The serum S100B concentrations of infants in the brain damage group was significantly elevated at age 7 days, 14 days, and 21 days, compared to the concentrations at the age of 3 days ($p < 0.05$). In addition, the S100B concentration was highest at age 14 days ($p < 0.05$); there was no significant difference between the data at age 7 days and 21 days ($p > 0.05$; Fig. 3).

Among all enrolled extreme preterm infants, most infants with brain damage had a serum S100B protein concentration higher than 1.0 μg/L and 1.5 μg/L at each age point (Fig. 3). Fig. 4 shows the ROC curve of serum S100B concentrations and brain damage. The AUC was as high as 0.985 ($p < 0.001$). The serum S100B cutoff value of 1.0 μg/L had a sensitivity of 93.8% and specificity of 90.5% in detecting brain damage, and the serum S100B cutoff value of 1.5 μg/L had a sensitivity of 84.4% and specificity of 98.3% in detecting brain damage.

4. Discussion

The present study demonstrated that the serum S100B concentration had a significantly positive correlation with ischemic brain damage in extreme preterm infants, and that serum S100B elevations could be detected earlier than by image findings using brain echography. These findings suggest that S100B may be a good early marker for diagnosing ischemic brain damage in extreme preterm infants. We also demonstrated that a S100B concentration higher than 1.0 μg/L had an elevated sensitivity and specificity in identifying brain damage earlier than brain imaging studies of extreme preterm infants with ischemic brain damage. To the best of our knowledge, this stated cutoff value is the first that has been reported in a published paper.

Noteworthy advances in the quality of intensive care of preterm newborns have increased their survival rate. However, the risk of cerebral injury with neurodevelopment abnormalities are becoming more common in extremely low birth weight preterm infants. Periventricular–intraventricular hemorrhage with venous infarct and PVL are the two most common problems of cerebral injuries in preterm infants. A diagnosis of white matter injury is usually obtained by noninvasive brain echography in most subclinical cases.[1] However, in infants with PVL, cystic lesions close to the lateral ventricles usually appear 2–3 weeks after birth. In addition, an increased periventricular echo density may persist for >14 days before cystic lesions are detected. When these echographic images are detected, brain damage has probably already occurred.

Periventricular leukomalacia consists of an ischemic infarction in the region of the cerebral white matter adjacent to the lateral ventricles; it is common in extremely preterm

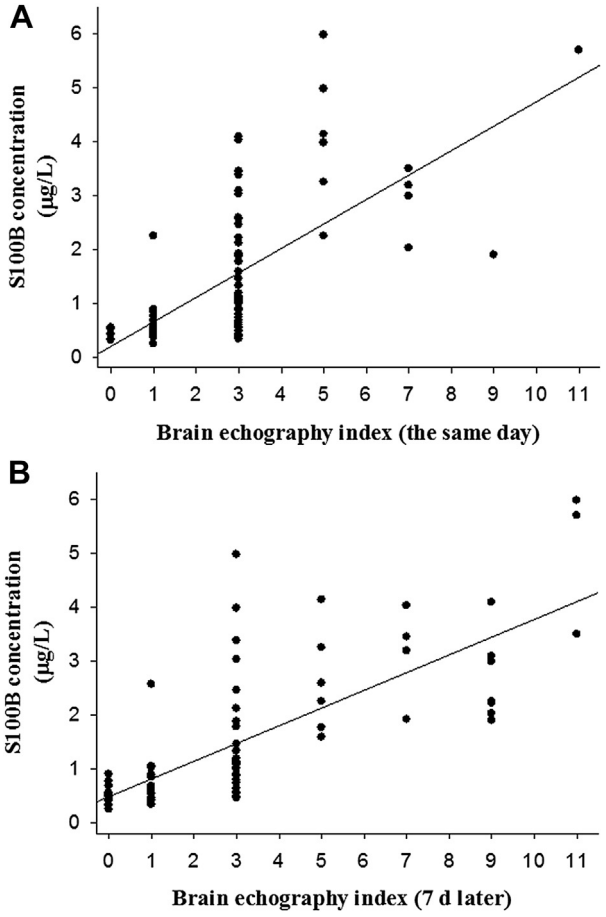

Fig. 2. The relationship between serum S100B levels and the brain echography index values of the study preterm infants. (A) Brain echography and the serum S100B level were performed on the same day (Spearman's correlation coefficient = 0.738; $p < 0.001$). (B) Brain echography index was calculated 7 days after blood was drawn to determine the serum S100B level (Spearman's correlation coefficient = 0.774; $p < 0.001$). S100B = S100 calcium-binding protein B.

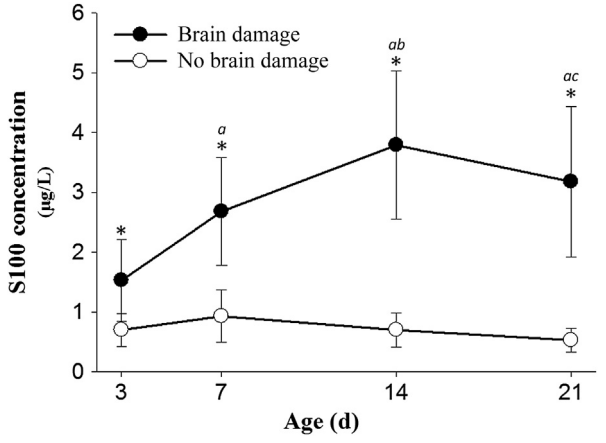

Fig. 3. The serum S100B levels in preterm infants with and without brain damage at the ages of 3 days, 7 days, 14 days, and 21 days. *$p < 0.05$ compared to the data of infants with no brain damage at the same age. [a] $p < 0.05$ compared to the data of the 3-day-old infants. [b] $p < 0.05$ compared to the data of the 7-day-old infants. [c] $p < 0.05$ compared to the data of the 14-day-old infants. S100B = S100 calcium-binding protein B.

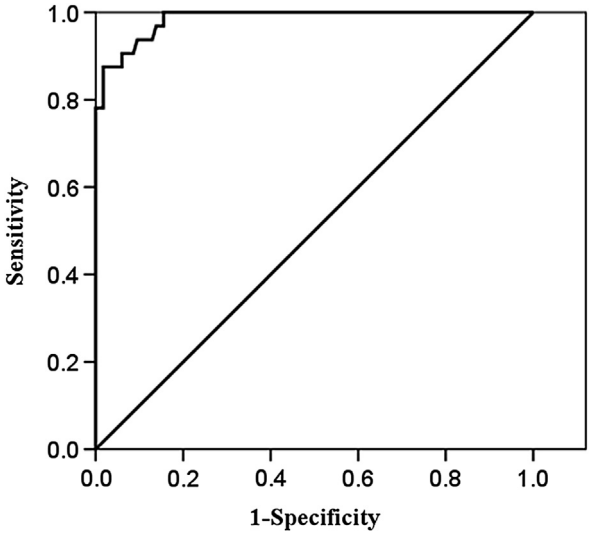

Fig. 4. The receiver operating characteristic (ROC) curve of serum S100B levels in predicting ischemic brain damage in study preterm infants [area under curve (AUC) = 0.985, $p < 0.001$]. The serum S100B protein level of 1.5 mg/L has a sensitivity of 84.4% and a specificity of 98.3%. The serum S100B protein level of 1.0 mg/L has a sensitivity of 93.8% and a specificity of 90.5%.

newborns with a gestational age of <32 weeks.[22] Microscopic changes of PVL are characterized by coagulation necrosis with microglia infiltration, astrocytic proliferation, decrease in the number of premyelinating oligodendrocytes, and eventual cystic formation.[23,24] These pathologic findings could explain the elevated S100B concentrations during ischemic injury in the white matter of preterm newborns because S100B is primarily concentrated in the glial cells of the nervous system and released when the cells are destroyed. When a cerebral injury occurs, the early response of the glial cells is to secrete S100B.[25] The high concentrations of S100B contribute to neuropathological changes through nitric oxide release, and activation of the caspase cascade with the resultant neural cell apoptosis.[26] For these reasons, S100B has a strong correlation with ischemic brain damage such as PVL.

Early elevation of S100B concentrations have been reported within hours in asphyxiated infants with severe brain damage and in infants with head injury.[11,13] Measuring the S100B concentration in conjunction with applying clinical decision rules in patients with minor head injury reportedly decreased the use of cranial computed tomography examinations by 30%.[27] The half-life of S100B is approximately 1 hour; therefore, a persistently elevated S100B concentration may imply sustained and extensive brain damage with the continuous release of S100B from damaged nerve tissue.[28] In our present brain damage group, elevated S100B concentrations persisted up to 21 days. This finding indicated that some ongoing changes existed in the patients' brain tissue. In addition, our study demonstrated a significant positive correlation between the BEI value and the serum S100B concentration of the tested infants, especially when examined 7 days later

(Fig. 2). Therefore, ischemic brain damage could be detected at least 7 days earlier by measuring the serum S100B concentrations than it would have been through brain echography. With respect to the earlier detection of brain damage by S100B in comparison to brain echography, the measurement of the S100B concentration when there is no defined lesion on echography is useful for instituting treatment strategies to prevent further cerebral damage.[29]

Fifteen years ago, Gazzolo et al[10] reported elevated serum S100B concentrations in preterm newborns with IVH. In addition, Distefano et al[30] demonstrated an early elevation of S100B protein concentration at 24 hours of life in preterm neonates with perinatal asphyxia; however, the values decreased 1–3 weeks later. In the present study, we demonstrated that elevated serum S100B concentrations could be detected in infants as young as 3 days old; the highest concentrations were noted at age 14 days in sick preterm newborns who later had significant image changes that resulted from PVL. Most cystic changes in the cranial echographic images in our study infants were evident at age 21 days or later. This finding may explain the reason for the highest S100B concentrations changes at 14 days of life. The different peak timetables of S100B concentrations between these two studies may be primarily attributable to the patients' perinatal condition, which was more pronounced in Distefano's patients.

The S100B concentration is reportedly correlated with an infant's gestational age.[31–33] Gazzolo et al[31] demonstrated an inverse relationship between the gestational age and the S100B concentrations in the cord blood of newborn infants; however, the investigators did not collect serial S100B concentrations or exclude preterm infants with abnormal brain images.

Our study grouped extreme preterm infants in accordance with their final brain image findings. The results showed smaller gestational ages and persistently higher S100B concentrations in the brain damage group. When we only focused on the well preterm infants, we found significantly lower S100B concentrations in the near-term infants than in the extreme preterm newborns. We also noted that all well infants, whether they were near-term or extreme preterm, had an S100B concentration of <1.0 μg/L. This finding is similar to the findings of other reports.[30] Therefore, using a serum S100B cutoff value of >1.0 μg/L for the early identification of brain damage in preterm infants may be reliable.

Severe hyperbilirubinemia may induce neurotoxicity and increase the release of S100B from the neuroglial cells. Preterm birth is a risk factor for hyperbilirubinemia.[30,34–36] Okumus et al[34] report a high correlation between the S100B concentrations and total bilirubin concentrations in term infants. Therefore, potential bilirubin encephalopathy should be considered when interpreting the serum S100B concentrations in newborn infants with hyperbilirubinemia. However, the proper assessment of bilirubin-induced neurotoxicity in premature infants is challenging.[36] Hyperbilirubinemia is common in high-risk preterm infants; therefore, neonatologists have paid much attention to it and treated it aggressively. A review of our enrolled patients showed that none of the study infants had sufficiently high bilirubin concentrations that required blood exchange transfusion or had any symptom of acute bilirubin encephalopathy.[37] Thus, bilirubin encephalopathy was not a confounding factor in the present study. However, future research to evaluate the relationship between hyperbilirubinemia, a biological marker, and the neurological outcome in extremely premature infants is important.

One limitation of the present study is that we only investigated the appearance of brain echographic images for ischemic brain damage without a long-term neurological follow up. Therefore, the elevation of S100B concentration to a concentration higher than 1.0 μg/L is only associated with the subsequent abnormal brain images and with not later neurological performance. Further study with long-term follow up for neurological development will be necessary for the future care of premature infants.

In conclusion**,** the serum S100B concentration is a useful biological marker to detect ischemic brain damage. Elevation of the serum S100B concentration has a high association with ischemic brain damage in extreme preterm infants. Therefore, ischemic brain damage is strongly suggested if the patient's early serum S100B concentration is higher than 1.0 μg/L in high-risk preterm infants.

Acknowledgments

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