The Serum S100B Concentration Is Age Dependent, *Luis V.C. Portela,[1,2] Adriano B.L. Tort,[1] Débora V. Schaf,[1] Luciana Ribeiro,[3] Daniel B. Nora,[3] Roger Walz,[4] Liane N. Rotta,[1,5] Cátia T. Silva,[6] João V. Busnello,[3] Flávio Kapczinski,[3] Carlos A. Gonçalves,[1] and Diogo O. Souza[1*]* ([1] Departamento de Bioquímica, ICBS, UFRGS, Porto Alegre, RS, 90035-003 Brazil; [2] Laboratório Municipal de Novo Hamburgo, RS, 93310-003 Brazil; [3] Departamento de Psiquiatria do Hospital de Clínicas de Porto Alegre, Porto Alegre, RS, 90035-003 Brazil; [4] Centro de Cirurgia de Epilepsia, CIREP, Hospital de Clínicas, FMRP-USP, Campus Universitário, Ribeirão Preto, SP, 14048-900 Brazil; [5] Laboratório de Patologia Clínica, HSL-PUCRS, Porto Alegre, RS, 91530-001 Brazil; [6] Serviço de Hemoterapia, Hemosul, Cachoeira do Sul, RS, 96508-970 Brazil; * address correspondence to this author at: Departamento de Bioquímica, ICBS, UFRGS, Rua Ramiro Barcelos 2600-Anexo, Porto Alegre, RS, 90035-003 Brazil; e-mail diogo@ufrgs.br)

S100B is a 21-kDa calcium-binding protein produced and released primarily by astrocytes in the central nervous system (CNS), where it exerts neurotropic and gliotropic actions *(1)*. Several studies have investigated the potential role of S100B as a peripheral biochemical marker of neural injury, including reactive gliosis, astrocytic death, and/or blood-brain barrier dysfunction *(2)*. Increased cerebrospinal fluid (CSF) and/or serum S100B has been reported in several acute and chronic injuries, including traumatic brain injury *(3)*, stroke *(4)*, Alzheimer disease *(5)*, schizophrenia *(6)*, HTLV-I-associated myelopathy *(7)*, and systemic lupus erythematosus *(8)*.

Previous neurodevelopmental studies have demonstrated age-related changes in S100B tissue expression and distribution in the CNS in mammals, which may be related to the different roles of this protein in distinct brain regions during the fetal period, adulthood, and aging *(1, 9)*. Furthermore, S100B concentrations in CSF have been noted to increase with age in healthy individuals *(10)*. Because these increases are more evident in men than women *(11)*, S100B concentrations in CSF may be sex and age dependent, suggesting that age- and sex-matched controls may be necessary for studies of S100B in CSF. To determine the usual concentrations for plasma S100B in adults, Wiesmann et al. *(12)* measured the protein in healthy blood donors and found no differences with sex and age in individuals 18–65 years of age, in agreement with our findings in previous studies in adults *(6–8)*.

To our knowledge, however, S100B concentrations in blood in childhood and adolescence were not established until recently. The present study was performed to verify S100B serum concentrations in healthy individuals of different ages, ranging from neonates to adults.

We collected blood samples from 19 healthy term neonates (<48 h of age) and from 25 healthy children 4–16 years of age undergoing routine clinical and laboratory evaluation. These children had no previous history of neurologic deficits or any other serious disorder, and their blood samples revealed no abnormal findings. Samples were also obtained from 85 healthy adults 18–70 years of age. This study was approved by local Ethics Committee of the Hospital de Clínicas de Porto Alegre (RS, Brazil). Informed consent was obtained from the adults and parents.

All blood samples were collected without anticoagulants by venipuncture. Serum was obtained by centrifugation at 3000*g* for 5 min and, soon thereafter, was frozen at −70 °C until analysis.

Serum S100B was measured using a monoclonal immunoluminometric assay (LIA-mat® Sangtec®100; Sangtec Medical) in a Lumat LB9507 luminometer (EG&G Berthold). Serum samples and calibrators (100 μL of each) were diluted with 100 μL of bovine serum albumin in test tubes provided with the assay. These tubes were already coated with anti-S100B antibody (three monoclonal antibodies: SMST 12, SMSK 25, and SMSK 28). After incubating for 1 h, the tubes were washed three times with wash buffer, and 200 μL of an antibody labeled with isoluminol as a tracer was added to each tube. After an additional 2-h incubation, during which this antibody bound to the immobilized S100B, the unbound tracer was washed out and the residual antibody was measured in a luminometer. S100B concentrations were derived by comparison with the calibration curve based on the total luminescence for each given calibrator provided with the assay. All determinations were carried out within the same experiment. The S100B calibration curve was linear up to 20 μg/L, and the CVs for duplicates across the entire concentration range for the calibrators and samples were <5%. The detection limit of the assay is 0.02 μg/L, as provided by the supplier of the LIA-mat Sangtec100 assay.

Statistical analyses were performed using the Mann–Whitney test for comparisons between sexes and the Spearman correlation coefficient to evaluate correlation between serum S100B and age. Comparisons of serum S100B concentrations among different age groups were performed by Kruskal–Wallis ANOVA, with Mann–Whitney post hoc testing. $P < 0.05$ was considered statistically significant.

No statistically significant difference was noted between serum S100B concentrations in male and female term neonates (age <48 h; $P = 0.60$), children (4–16 years; $P = 0.59$), adults (18–70 years; $P = 0.18$), or in all age groups combined ($P = 0.67$). We found a negative correlation between S100B and age (Fig. 1; $r = -0.70$; $P < 0.001$), with the higher concentrations in neonates, as shown in the inset of Fig. 1. Moreover, a correlation between age and S100B was not evident in individuals >20 years of age (21–70 years; n = 76; $r = -0.05$; $P = 0.76$), but was present in individuals <20 years of age (0–20 years; n = 53; $r = -0.87$; $P < 0.001$). Table 1 shows the medians and interquartile ranges obtained for the various age groups.

In agreement with the results obtained by Wiesmann et al. *(12)*, we also found no sex dependence in serum S100B concentrations in adults. In addition, our data revealed no sex-related difference in S100B concentrations in the blood of neonates and children. Importantly, when we com-

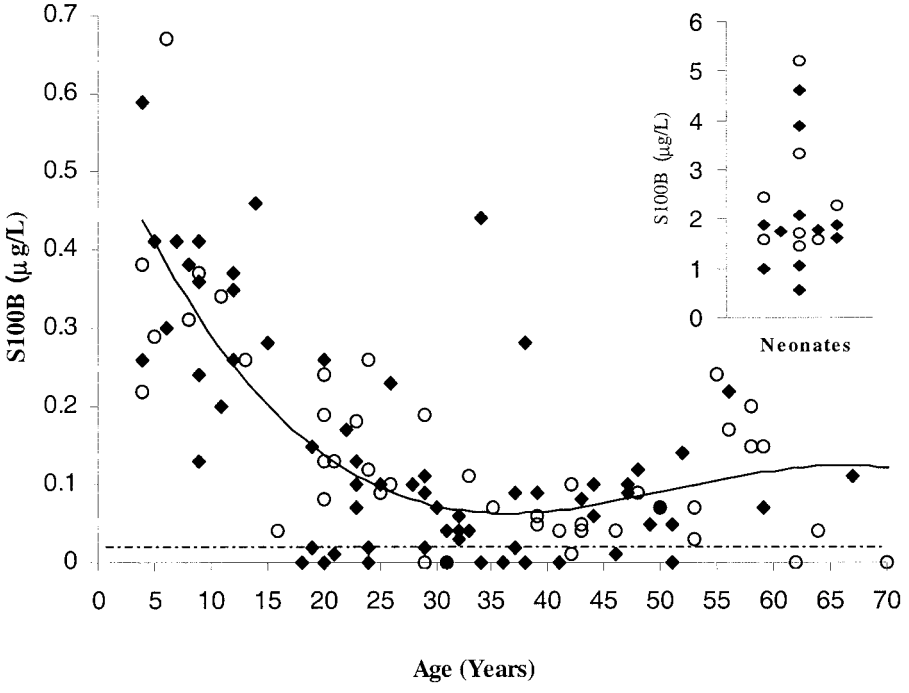

Fig. 1. Correlation of serum S100B protein concentrations with age.

♦, males; ○, females. *Inset* shows serum S100B in term neonates. A statistically significant negative correlation was observed ($r = -0.70$; $P < 0.001$). The fitted curve (*solid line*) was obtained using a third-degree polynomial regression. The *dashed line* represents the detection limit of the assay (0.02 μg/L).

pared serum S100B in all individuals with their corresponding age, we found a statistically significant negative correlation ($r = -0.70$; $P < 0.001$).

Only a few studies have been published on the determination of S100B in the blood of neonates and children *(13–16)*. Although these previous studies did not attempt to correlate S100B concentrations in the blood with age, their results suggested that the baseline blood concentrations in neonates and young infants could be higher than in adults. Our results suggest that S100B concentrations in term neonates are much higher than in older individuals. It is worth pointing out that bilirubin up to 150 mg/L did not interfere with the S100B assay (data not shown). On the basis of our data, blood S100B concentrations appear to decrease considerably in the first two decades of life ($r = -0.87$; $P < 0.001$) and remain relatively constant during adulthood ($r = -0.05$; $P = 0.76$), which is in accordance with the results obtained by Wiesmann et al. *(12)*.

Table 1. Serum S100B in the different age groups.

Group	Age, years	n	S100B, μg/L Median	S100B, μg/L Interquartile range[a]	Differences among groups Compared with groups	Differences among groups *P*
1	Neonates	19	1.790	1.570–2.440	2–7	<0.001
2	4–9	16	0.365	0.267–0.410	3	0.35
					4–7	<0.001
3	10–15	8	0.310	0.260–0.365	4–7	<0.001
4	16–20	10	0.105	0.015–0.202	5	0.68
					6	0.97
					7	0.383
5	21–25	13	0.100	0.045–0.150	6	0.84
					7	0.09
6	26–30	9	0.100	0.055–0.150	7	0.16
7	30–70	54	0.060	0.030–0.100		

[a] 25th–75th quartiles.

Several theories can be offered to explain the results obtained in our study. The higher concentrations of serum S100B protein found in neonates and children compared with adults could reflect the ongoing central neurodevelopmental processes occurring during these different stages of life *(9)*. The proliferation and maturation of glial cells, extension of neurites, and formation of synapses have been documented to be the most important functions of S100B in morphogenesis *(1, 17, 18)*, possibly involving mitogen-activated protein kinase *(19)* and activation of receptors for advanced glycation end products in neurons *(20)*. It is also interesting to note that some nerve fibers are positive for S100B during development, implicating a possible role for S100B in regulating the activity of fiber sprouting *(9)*. Therefore, S100B, like other glial-derived cytokines, may be involved in coordinating the development and maintenance of the CNS by synchronously stimulating the differentiation of neurons and the proliferation of astroglia.

Functional maturation of the human brain has been studied with positron emission tomography, and the regional or local cerebral metabolic rates of glucose utilization (LCMRglc) have been used as indirect measurement of synaptogenesis in the human brain *(21, 22)*. These studies showed that the LCMRglc in the cerebral cortex of children between the ages of ~4 and 9–10 years exceed adult rates by more than twofold. After 9–10 years, LCMRglc for cerebral cortex begin to decline and gradually reach adult values by 16–18 years. In addition, magnetic resonance imaging has shown an increase of

gray matter during childhood, which decreases before adulthood *(23)*. From the electrophysiologic point of view, electroencephalographic findings become similar to those in adults early in the second decade of life *(24)*.

The time window of maximal plateau and decline of LCMRglc in cerebral cortex and the consolidation of adult electroencephalographic patterns observed in older children are closely associated with the variations in serum concentrations of S100B in healthy individuals. Considering that the S100B protein exerts tropic effects on neural cells, we believe that it could play an important role during the early phase of human brain development, which is reflected in its serum concentrations.

In conclusion, we have shown a negative correlation between blood S100B and age in the first 20 years of life; after age 20, S100B does not appear to vary with age. Therefore, in studies involving measurement of blood S100B in pediatric and adolescent patients, it is important to establish age-matched reference values. Further work will be required to clarify the role of S100B during neural development.

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References

1. Donato R. S100: a multigenic family of calcium-modulated proteins of the EF-hand type with intracellular and extracellular functional roles. Int J Biochem Cell Biol 2001;33:637–68.
2. Wong CH, Rooney SJ, Bonser RS. S100β release in hypothermic circulatory arrest and coronary artery surgery. Ann Thorac Surg 1999;67:1911–4.
3. Ingebrigtsen T, Waterloo K, Jacobsen EA, Langbakk B, Romner B. Traumatic brain damage in minor head injury: relation of serum S-100 protein measurements to magnetic resonance imaging and neurobehavioural outcome. Neurosurgery 1999;45:468–75.
4. Wunderlich MT, Ebert AD, Kratz T, Goertler M, Jost S, Herrmann M. Early neurobehavioural outcome after stroke is related to release of neurobiochemical markers of brain damage. Stroke 1999;30:1190–5.
5. Sheng JG, Mrak RE, Rovnaghi C, Kozlowska E, Van Eldik LJ, Griffin WS. Human brain S100B and S100B mRNA expression increases with age: pathogenic implications for Alzheimer's disease. Neurobiol Aging 1996;17:359–63.
6. Lara DR, Gama CS, Belmonte-de-Abreu P, Portela LVC, Gonçalves CA, Fonseca M, et al. Increased serum S100B protein in schizophrenia: a study in medication-free patients. J Psychiatr Res 2001;35:11–4.
7. Walz R, Portela LVC, Tort ABL, Neto EC, Fernandes LN, Gonçalves CA, et al. Serum S100B levels in patients with HTLV-I associated myelopathy/tropical spastic paraparesis. Neurology 2000;54:2021–2.
8. Portela LV, Brenol JC, Walz R, Bianchin M, Tort AB, Canabarro UP, et al. Serum S100B levels in patients with lupus erythematosus: preliminary observation. Clin Diagn Lab Immunol 2002;9:164–6.
9. Tiu SC, Chan WY, Heizmann CW, Schäfer BW, Shu SY, Yew DT. Differential expression of S100B and S100A6 in the human fetal and aged cerebral cortex. Dev Brain Res 2000;119:159–68.
10. Van Engelen BG, Lamers KJ, Gabreels FJ, Wevers RA, van Geel WJ, Borm GF. Age-related changes of neuron-specific enolase, S-100 protein, and myelin basic protein concentrations in cerebrospinal fluid. Clin Chem 1992;38:813–6.
11. Nygaard Ø, Langbakk B, Romner B. Age- and sex-related changes of S-100 protein concentrations in cerebrospinal fluid and serum in patients with no previous history of neurological disorder. Clin Chem 1997;43:541–3.
12. Wiesmann M, Missler U, Gottmann D, Gehring S. Plasma S-100b protein concentration in healthy adults is age- and sex-independent. Clin Chem 1998;44:1056–7.
13. Gazzolo D, Vinesi P, Marinoni E, Di Iorio R, Marras M, Lituania M, et al. S100B protein concentrations in cord blood: correlations with gestational age in term and preterm deliveries. Clin Chem 2000;46:998–1000.
14. Gazzolo D, Bruschettini M, Lituania M, Serra G, Gandullia E, Michetti F. S100b protein concentrations in urine are correlated with gestational age in healthy preterm and term newborns. Clin Chem 2001;47:1132–3.
15. Ashraf S, Bhattacharya K, Tian Y, Watterson K. Cytokine and S100B levels in paediatric patients undergoing corrective cardiac surgery with or without total circulatory arrest. Eur J Cardiothorac Surg 1999;16:32–7.
16. Amer-Wahlin I, Herbst A, Lindoff C, Thorngren-Jerneck K, Marsal K, Alling C. Brain-specific NSE and S-100 proteins in umbilical blood after normal delivery. Clin Chim Acta 2001;304:57–63.
17. Marshak DR. S100β as a neurotrophic factor. Prog Brain Res 1990;86:168–81.
18. Selinfreud RH, Barger SW, Pledger WJ, Van-Eldik LJ. Neurotrophic protein S100β stimulates glial cell proliferation. Proc Natl Acad Sci U S A 1991;88:3554–8.
19. Gonçalves DS, Lenz G, Karl J, Gonçalves CA, Rodnight R. Extracellular S100B protein modulates ERK in astrocyte cultures. Neuroreport 2000;11:807–9.
20. Huttunen HJ, Kuja-Panula J, Sorci G, Agneletti AL, Donato R, Rauvala H. Coregulation of neurite outgrowth and cell survival by amphoterin and S100 proteins through receptor for advanced glycation end products (RAGE) activation. J Biol Chem 2000;275:40096–105.
21. Chugani HT, Phelps ME, Mazziota JC. Positron emission tomography study of human brain functional development. Ann Neurol 1987;22:487–97.
22. Chugani HT. A critical period of brain development: studies of cerebral glucose utilization with PET. Prev Med 1998;27:184–8.
23. Durston S, Hulshoff Pol HE, Casey BJ, Giedd JN, Buitelaar JK, Van Engeland H. Anatomical MRI of the developing human brain: what have we learned? J Am Acad Child Adolesc Psychiatry 2001;40:1012–20.
24. Niedermeyer E. Maturation of the EEG: development of waking and sleep patterns. In: Niedermeyer E, Lopes da Silva F, eds. Electroencephalography. Basic principles, clinical applications, and related fields, 4th ed. Philadelphia: Lippincott Williams & Wilkins, 1999:189–214.

"Reconstituted" α-Thalassemia Genomic Samples as Positive Controls for the Molecular Diagnostic Laboratory, *Wen Wang,[1] Arnold S-C. Tan,[1] and Samuel S. Chong[1,2,3*]* ([1] Departments of Pediatrics and Obstetrics and Gynecology, National University of Singapore, Singapore 119074, Singapore; [2] Molecular Diagnosis Center, Department of Laboratory Medicine, National University Hospital, Singapore 119074, Singapore; [3] Department of Pediatrics, The Johns Hopkins University School of Medicine, Baltimore, MD 21205; * address correspondence to this author at: Department of Pediatrics, National University of Singapore, Level 4, Main Building, National University Hospital, 5 Lower Kent Ridge Rd., Singapore 119074, Singapore; fax 65-6779-7486, e-mail paecs@nus.edu.sg)

We and others recently described strategies for multiplex-PCR analysis of deletional determinants of α-thalassemia *(1–3)*, culminating in the development of a single-tube assay for simultaneous screening of seven common deletions *(4)*. Since then, several molecular diagnostic laboratories wanting to set up the test have requested DNA samples carrying these deletions for use as validation and positive controls. These requests have led to a critical shortage of our limited stocks of genomic samples, especially those with the rarer deletions, something that has caused our inability to fulfill all requests. The ideal solution to limited genomic DNA is to establish immortal lymphoblastoid cell lines from peripheral-blood leukocytes of patients by Epstein–Barr virus transformation *(5, 6)*. To do so, however, requires that patients be contacted again to provide renewed consent and a fresh aliquot of blood for transformation, something that may