observed between serum and heparin-treated plasma. Thus, sodium citrate- and EDTA-treated samples may yield more accurate values for cystine.

In summary, the anticoagulant used in sample preparation may markedly affect the results of amino acid analysis by automated ion exchange chromatography. Sample preparation should be standardized, and clinicians should be aware of the possible sources of error. We recommend the use of either heparin or EDTA exclusively, with careful attention to potential inaccuracies in cystine concentrations with the former and PTEN with the latter.

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References

Plasma S-100b Protein Concentration in Healthy Adults Is Age- and Sex-Independent, Martin Wiesmann,1* Ulrich Missler,1 Daniela Gottmann,1 and Svante Gehringer2 1 Neuroradiology, Klinikum Grosshadern, Ludwig-Maximilian University, 81377 Munich, Germany, and 2 Immunology and Transfusion Medicine, University of Luebeck, School of Medicine, 2400 Luebeck, Germany; *address for correspondence: Abteilung fuer Neuroradiologie, Klinikum Grosshadern, Ludwig-Maximilians-Universitaet, Mar-chioninstr. 15, 81377 Muenchen, Germany; fax 0049 (89) 7095 3270, e-mail wiesmann@ikra.med.uni-muenchen.de

S-100 protein (S-100) is a calcium-binding protein found predominantly in the cytosol of glial cells in all parts of the central nervous system (CNS). Three different subtypes, designated S-100a, S-100b, and S-100a0, are known. S-100b predominates in the brain.

The concentration of S-100 can be measured in cerebrospinal fluid (CSF) and in blood. For methodological reasons, most studies reported in the literature measured S-100 concentrations in CSF, and an increased concentration of S-100 in the CSF has been found to be a sensitive although nonspecific indicator of nervous system damage in patients with various neurological disorders (1).

Increased concentrations of S-100 have also been found in the blood of patients suffering from CNS tumors or cerebrovascular insults, and maximal concentrations of S-100 in the blood are correlated with the infarct volume after acute ischemic stroke (1–6). It has recently been reported that concentrations of S-100 in blood may be of prognostic value in patients with minor head injury (7) and may indicate acute exacerbation of multiple sclerosis (8).

Van Engelen et al. (9) reported that S-100 concentrations in CSF increase with age. Nygaard et al. (10) confirmed this and also found a difference in the mean concentration of S-100 in the CSF of male and female subjects. For routine clinical use, these findings would necessitate age- and sex-corrected reference intervals.

We have described a method for determining the concentration of S-100 in blood that is sensitive enough to detect the concentration of S-100 in the plasma of healthy subjects (1). We believe that this method could be useful clinically, but the dependancy on age or sex of S-100 concentrations in blood has not been studied yet. We therefore conducted a study to determine whether S-100 concentrations in blood change with age or depend on the individual’s sex.

Blood samples were obtained from 200 healthy blood donors between 18 and 65 years of age who had no history of previous neurological deficit or any other serious disorder. Subjects were receiving no medications, and the results of physical examination and routine laboratory tests were normal. The study was in accordance with the current revision of the Helsinki Declaration of 1975, and all subjects gave informed consent to the procedure.

Subjects were grouped according to age: 18–25 years, 26–35 years, 36–45 years, 46–55 years, and 56–65 years. Each group was composed of 40 individuals, 20 men and 20 women. Blood was collected in heparin-containing tubes, centrifuged within 12 h of collection, and stored at –70 °C until analysis.

S-100 concentrations were determined with an immunofluorometric sandwich assay as described earlier (1). In brief, all measurements were set up in duplicate. Microtiter plates coated with 10 μL of anti-S-100 β-chain (Sigma Chemical) in 20 mL of phosphate buffer (0.05 mol/L, pH 8.6) were incubated with 200 μL per well of S-100 calibrator, controls, or samples for 120 min. Biotin-labeled rabbit anti-S-100 antibody (DAKO) in a Tris (0.05 mol/L), NaCl (0.15 mol/L), CaCl2 (10 mmol/L), NaN3 (0.15 mmol/L) buffer was added, and the plates were incubated for another hour. After the plates had been washed, 200 μL of streptavidin-europium in assay buffer (0.05 mol/L Tris; 0.15 mol/L NaCl; 1 g/L bovine serum albumin; 0.5 g/L bovine γ-globulin, both from Sigma; and 0.15 mmol/L NaN3) was added to each well, and the plates were incubated for 30 min. As a last step, 200 μL of enhancement solution (0.01 mol/L acetic acid, 38 mg/L tri-n-octylphosphine oxide, 1.3 g/L potassium phthalate,
222 mg/L thenoyltrifluoroacetone, and 2 mL/L Triton X-100) was added to each well, and the plates were incubated for 15 min. The resulting fluorescence was measured with a DELFIA 1234 fluorometer (Wallac). The threshold for detection of S-100 with this assay was 0.015 μg/L.

For each age group, the median concentration of S-100 and the 25th, 75th, 10th, and 90th percentiles of S-100 concentration were calculated. The significance of differences in median concentration between groups was examined using the Mann–Whitney U-test, and the correlation between age and S-100 concentration was calculated using the Spearman rank order correlation coefficient.

For all 200 healthy volunteers, the median plasma concentration of S-100b was 0.052 μg/L (10th percentile, 0.023 μg/L; 90th percentile, 0.097 μg/L; Fig. 1). The difference in median concentrations for men (median, 0.055 μg/L) and women (median, 0.048 μg/L) was not statistically significant (P >0.05). With increasing age, plasma concentrations of S-100b decreased slightly. The correlation between decreasing concentration and increasing age was weak, however, (r = −0.144; P = 0.04), and differences between age groups were not significant (P >0.05; Fig. 1).

The possibility that the concentration of S-100 in CSF might be age-dependent is based on two published reports. Van Engelen et al. (9) studied S-100 concentrations in the CSF of patients who were undergoing neurological examination but who had no evidence of an organic neurological disease. That study found that the concentration of S-100 increased slightly with increasing age but did not differ substantially by sex. Nygaard et al. (10) examined patients undergoing various surgical procedures with spinal anesthesia. That study found substantially higher concentrations of S-100 in the CSF with increasing age. They also found that S-100 concentrations in the CSF were substantially higher in men than in women. Both of these groups used analytical methods that detected predominantly S-100b but were not sensitive enough to detect S-100 concentrations in the blood of healthy subjects.

Several explanations have been offered as to why the concentration of S-100 in CSF might increase with increasing age: (a) the concentration of S-100 in CSF might parallel the rate of myelin loss, which increases with age; (b) the turnover of CNS cells remains constant, but S-100 concentrations in the cells increase with age; and (c) a reduced CSF bulk flow with older age leads to an increased half-life of S-100 in CSF (10).

In contrast to these findings regarding S-100 concentrations in CSF, our study found a slight decrease in the S-100b concentration in blood with increasing age, although the differences between age groups were not significant. The trend toward decreasing concentrations of S-100b with increasing age was mainly because of the finding of relatively high concentrations of S-100b in a few young individuals. Eight of the 10 subjects with the highest concentrations of S-100b were younger than 30 years. We found nothing in the data that would explain this finding.

The concentration of S-100 in blood may be useful clinically to screen for and monitor progression of damage to the CNS. Our study found no evidence that age- and sex-corrected reference values need to be established for measurements of S-100 concentrations in the blood of adults. Such may not be the case, however, for determinations of S-100 concentrations in children because the results of studies in animals indicate that S-100 concentrations in the brain change substantially during postnatal development of the brain (11). Therefore, reference values for different age groups of children should be established before this method is used routinely to evaluate acute brain damage in pediatric patients.

References
S100 Blood Concentrations in Children Subjected to Cardiopulmonary By-Pass, Diego Gazzolo, Paola Vinesi, Maria Concetta Geloso, Carlo Marcelletti, Fiore S. Iorio, Adriano Cipriani, Stefano M. Marianeschi, and Fabrizio Michetti (1 Dept. of Child Health and Neonatal Medicine, Giannina Gaslini Children’s Hospital, 16147 Genoa, Italy; 2 Institute of Histology Catholic University, 00168 Rome, Italy; 3 Institute of Anatomy, University of Bari, 70124 Bari, Italy; 4 Heart Surgery Service, Quisisana Hospital, 00197 Rome, Italy; *author for correspondence: Institute of Histology, Catholic University, Largo F. Vito, I, 00168 Rome, Italy; fax 39 6 3051343, e-mail ibis@rm.unicatt.it)

S100 is a member of a family of calcium-binding proteins present primarily in nervous tissue, where it is mainly concentrated in glial cells (1, 2). Although the role of this protein in brain function and disease has not yet been conclusively elucidated, it has been ascertained that the appearance of this protein constituent of neural cells in biological fluids is a reliable indicator of active cell damage in the nervous system in different pathological conditions (3–6).

Open heart surgery is known to be associated with brain cell injury. It was diagnosed previously using rather crude psychometric tests and clinical observations in seemingly healthy patients (7), and measurements of S100 in the blood have recently been successfully used to monitor cerebral damage after cardiac surgery (8–10).

The present study is aimed at investigating the potential use of measurements of S100 in the blood to monitor possible cerebral distress during extracorporeal circulation in children (0–9 years), when hemodynamic adaptive phenomena in the brain show peculiarities linked to the age of the patient (11). The data presented indicate significantly higher blood concentrations of this nervous tissue constituent during cardiopulmonary by-pass (CPB), together with a direct relationship with the duration of CPB.

Blood samples were taken from 13 patients (6 males and 7 females), ages from <1 year to 9 years (two patients <1 year of age, one 1 year of age, six 3 years of age, one 4 years of age, one 5 years of age, and two 9 years of age), with no preexisting neurological disorders or other comorbidities, who were undergoing cardiac surgery with CPB for correction of congenital heart disease without emergency procedure. The samples were taken at five predetermined times before, during, and after surgery (time 0, before surgery; time 1, during surgery before CPB; time 2, at the end of CPB; time 3, at the end of surgery; time 4, 12 h after surgery) and measured for S100. Clinical parameters (peripheral temperature, nasopharyngeal temperature, pump flow rate, mean blood pressure, and central and peripheral blood pH) were recorded at all sample times to monitor the general pattern of surgery. Eleven patients had satisfactory postsurgery follow-up, but two patients, whose preoperative general condition was not appreciably different from the others, died as a result of heart failure 36 and 72 h after surgery. The study protocol was approved by the Ethics Committee of Quisisana Hospital, Rome.

Heparin-treated blood samples were immediately centrifuged at 900g for 10 min, and the supernatants were stored at −70°C before measurement. The S100 protein concentrations were measured in all samples, using a commercially available two-site IRMA (Sangtec 100, AD Sangtec Medical).

Each measurement was performed in duplicate. Either 100 μL of S100 in known concentrations (ranging between 0.5 and 60 μg/L) or 100 μL of patient sample were added to each tube. One hundred microliters of Sangtec 100 IRMA diluent and one plastic bead coated with monoclonal anti-S100 antibody were then added to each tube. The tubes were incubated for 1 h at room temperature (18–20°C) on a shaker. Each bead was then washed once with 2 mL of demineralized water, and 200 μL of tracer (125I-labeled monoclonal anti-S100 antibody) was added to each tube. To estimate total counts, 200 μL of tracer was placed in a tube, and the radioactivity was then counted without additional processing. The tubes were incubated for 2 h at room temperature on a shaker. Each bead was then washed three times with 2 mL of demineralized water each time. The radioactivity was counted in a gamma counter for 60 s. The amount of S100 in the sample was then calculated using a standard curve prepared with calibrators with known concentrations of S100. S100 blood concentrations <0.5 μg/L were considered undetectable.

The data were analyzed by the Wilcoxon paired two-sided test and by linear regression analysis. A value of \( P < 0.05 \) was considered as significant.

The concentrations of S100 in the blood of patients with favorable outcomes were undetectable or at the detection limit before sternotomy in patients successfully overcoming surgery and the postoperative period (Fig. 1A), in accordance with previous reports using the same procedure to measure blood S100 (12). The concentrations increased during surgery, with the highest S100 values being detected at the end of CPB (time 2), when they were significantly higher (\( P < 0.01, n = 11 \)) than before surgery. Post-CPB S100 values decreased to concentrations not significantly different from those recorded before surgery. The two patients who died after surgery had the highest S100 concentrations during and after the surgical procedure and also exhibited detectable S100 concentrations before surgery.