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CORRELATION OF SERUM S100B CONCENTRATION WITH HOSPITAL STAY IN PATIENTS UNDERGOING CABG

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SUMMARY – S100B protein has been proposed to be a serum marker of cerebral injury in patients undergoing cardiac surgery. The question to be answered in the present study was whether an increase in serum S100B concentration after the surgery correlated with the length of hospital stay in patients undergoing coronary artery bypass grafting. To answer this question we measured serum S100B concentration preoperatively, at the end of the operation, and on day 1 and day 5 of the surgery in 32 patients undergoing coronary artery bypass grafting. The median (min; max) hospital stay was 7 days (5; 34), and serum S100B concentration was 0.075 mg/L (0.050;0. 095) preoperatively, 0.840 mg/L (0.390; 1.500) immediately after the operation, 0.180 mg/L (0.150;0. 280) on day 1 and 0.100 mg/L (0.080;0. 120) on day 5 of the operation. None of the patients had clinical signs of cerebral injury. Multivariate linear regression analysis indicated serum S100B concentration on day 1 (p=0.0296) and day 5 (p=0.0021) of the operation to correlate with the length of hospital stay independently of the type of operation (with or without the use of cardiopulmonary bypass) and patient clinical characteristics. Our data suggest that serum S100B concentration on day 1 and day 5 of the operation may have prognostic value in patients without clinical signs of cerebral injury. However, this pilot study should be extended to a larger group of patients to confirm this observation.

Key words: *Coronary artery bypass – adverse effects; Coronary artery bypass – methods; S100 proteins – blood; Biological markers – blood*

Introduction

Cerebral injury persists as the predominant postoperative complication in patients undergoing coronary heart surgery. The incidence of stroke is 1.6%[1]. Off-pump coronary artery bypass grafting (CABG) has reduced postoperative neuropsychological dysfunction in elderly patients with severe systemic atherosclerosis compared to on-pump CABG. The incidence of neuropsychological dysfunction was 11.2% in the off- pump group and 22.5% in the on-pump group[2]. S100B protein has been shown to be a sensitive marker of cerebral injury during and after cardiac operations. For instance, S100B released after 5 to 48 hours was associated with cerebral complications and risk factors for such outcome[3]. A greater number of cerebral microemboli were found in patients undergoing on-pump as compared to off-pump CABG[4]. Furthermore, increased S100B in patients with stroke following cardiac surgery correlates with the size of infarcted brain tissue and has a negative predictive value for median term survival[5]. Finally, CABG with cardiopulmonary bypass (CPB) caused a 10-fold increase in S100B recorded in off-pump grafting[6].

The S100B is a small calcium binding protein (21-kD), which exists in several isoforms. The S100-αβ and -ββ forms are predominantly present in astroglial or microglial cells, and are considered to be highly specific for the brain[7]. Clinical researches and researches on different animal models point to important pathophysiological

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role of S100B in some diseases. For instance, increased concentrations have been found in Down syndrome and some forms of epilepsy, which could be the consequence of increased expression of the gene for S100B that is otherwise positioned on chromosome 21[8-10].

The aim of the present study was to determine whether increased serum S100B concentration correlated with the length of hospital stay in patients undergoing CABG. This may be of some importance when evaluating justifiability of routine S100B measurement after cardiac surgery.

Subjects and Methods

Subjects

Thirty-two patients were consecutively enrolled among patients with ischemic heart disease undergoing routine CABG. Sixteen patients were operated on by use of CPB and the other 16 patients were operated on without it. Inclusion criteria were as follows: no neurologic dysfunction prior to the surgery and no renal impairment as verified by normal serum creatinine concentration (<97 mmol/L) before surgery. Blood samples were collected preoperatively, immediately after the operation, and on day 1 and day 5 of the operation. The study was approved by the ethics committee.

Operation

General anesthesia and then median sternotomy were performed in all patients. Left internal mammary artery or peripheral veins were dissected. In the off-pump group, 1 mg/kg heparin was administered and then anastomoses were created. Lima stitch, Octopus 2 stabilizer, blower device and cell saver were used. In the group with extracorporeal circulation, 3 mg/kg heparin was administered and the aorta and right atrium were cannulated with 24 Fr arterial perfusion catheter and DLP two-stage venous cannula. A heart-lung machine was connected and modified continuous flow was maintained between 2.2 and 2.6 mL/m^2/min. A hollow fiber oxygenator and arterial filters with 30- or 40-μm pores were used. Priming solution consisted of 2 L Ringer lactate, 50 mg heparin, 250 mL mannitol and 1 g methylprednisolone. Cardioplegia with 6° C, which consisted of oxygenated blood and crystalloid cardioplegic solution mixed at 1:4 ratio, was delivered antegradely and then repeatedly retrogradely every 10 minutes. The minimal patient rectal temperature was 33 °C. Topical cooling was used in few cases. Then, all the anastomoses were created. The last reperfusion with either cardioplegic solution or only oxygenated blood was normothermic. The mean CPB time was 126±34 (range 78-197) min and mean aortic cross clamp time was 76±22 (range 46-131) min. Prior to the removal of arterial cannula, protamine sulfate was given in the same dose as heparin.

Laboratory analysis

After centrifugation and separation of blood cells, serum samples were stored frozen at -20 °C. S100B concentration was determined by the luminometric immunoassay and AB Sangtec Medical analyzer (Bromma, Sweden)[11] in one batch at the end of the study. Imprecision calculated as coefficient of variation was 6.4% at serum S100B concentration of 0.33 mg/L and 5.0% at 3.88 mg/L.

Statistical analysis

Mean and standard deviation is presented in case of normally distributed variables, and median and 25th and 75th percentiles in case of non-normally distributed variables. Associations between variables were analyzed by calculating Pearson's correlation or Spearman's rank order correlation coefficient. Forward and backward multivariate linear regression analysis in a stepwise manner was used to study associations of hospital stay with other variables observed (F to enter model 3.5, F to remove 3.0 and tolerance 0.05). Logarithmic transformation of non-normally distributed variables was made before linear regression analysis to reduce the influence of outliers and to fit the linear model. All statistical analyses were done with the STATISTICA/Win program package of StatSoft, Inc. (Microsoft Corporation, Tulsa, OK, USA). Values of $p<0.05$ were considered to indicate statistical significance.

Results

Clinical characteristics of study patients are summarized in Table 1. Sixteen of 32 patients were operated on by use of CPB and the rest (16 patients) without it. Both groups were homogeneous according to age and body mass index (BMI). Females were more frequently operated on with the use of CPB. Hospital stay (days) was shorter in patients operated on without the use of CPB as compared to the other group.

Table 1. Patient clinical characteristics and laboratory findings

	All patients (N =32)	Patients with CPB	Patients without CPB
Sex (female/male)	5/27	5/11	0/16*
Age (yrs)		62±12 (29-80)	59±9 (41-71)
Body mass index (kg/m^2)		26.5±2.94(20.5-32.1)	28.8±3.5(23.7-34.0)
Creatinine preoperatively (μmol/L)		78.8±12.6 (65-117)	93.7±14.3(78-130)***
Hospital stay (days)	7 (5; 34)	9 {7.0/13.0}(7-34)	7 {5.5/7.5}(5-10)***
S100B preoperatively (μg/L)	0.075 (0.050;0.095)	0.08 {0.06/0.11} (0.04-0.30)	0.06 {0.04/0.08} (0.02-0.45)
S100B immediately after operation (μg/L)	0.840 (0.390;1.500)	1.53 {0.99/2.32} (0.65-4.48)	0.41 {0.26/0.55}**** (0.20-1.75)
S100B day 1 of operation (μg/L)	0.180 (0.150;0.280)	0.28 {0.16/0.41} (0.15-1.96)	0.15 {0.11/0.18}** (0.06-0.40)
S100B day 5 of operation (μg/L)	0.100 (0.080;0.120)	0.11 {0.09/0.20} (0.05-1.77)	0.08 {0.07/0.12} (0.03-0.17)

CPB = cardiopulmonary bypass; values are median ± SD (range) or median {25th percentile/75th percentile} (range) or number of participants; levels of significance: *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001

Laboratory findings are summarized in the second part of Table 1. There was no difference in serum S100B concentration between patient groups before the operation. Four of 16 patients operated on with the use CPB had serum S100B concentration above the upper reference value (0.15 mg/L) but below 0.30 mg/L. In the group of patients operated on without CPB, only one had serum S100B value above the upper reference value, i.e. 0.45 mg/L. Immediately after the operation, serum S100B concentration increased significantly (on an average 19-fold concentration before the operation in patients operated on with CPB and 7-fold in patients operated on without CPB) and was highest in the entire period of follow up. The highest serum S100B concentration was more than 30-fold the upper reference value. The time pattern of serum S100B concentration differed between the two patient groups. The patients operated on with the use of CPB had a significantly higher concentration immediately after and day 1 of the operation. There was no between-group difference in serum S100B concentrations measured before and day 5 of the operation.

Linear regression analysis revealed that the length of hospital stay did not correlate with age and sex (Table 2). On the contrary, hospital stay correlated significantly with the type of operation (p=0.0002) and serum S100B concentration before (p=0.0316), immediately after (p=0.0008), day 1 (p=0.0006) and day 5 (p=0.0036) of the operation (Table 2).

Multivariate linear regression analysis revealed (Table 3) that age, type of operation and serum S100B concentration were the most powerful parameters indicating the length of hospital stay on day 1 (model B1) and day 5 (model C1) of the operation. In order to establish whether serum S100B concentration independently pre-

Table 2. Correlation of hospital stay with type of operation and serum S100B concentration

Parameter	Spearman (r_s)
Dependent variable:	
Hospital stay (days)	
Independent variables:	
Sex (female/male)	–0.2971
Age (yrs)	+0.2680
Creatinine preoperatively (μmo/L))	–0.3166
Operation (type)	–0.6138***
S100B preoperatively (μg/L)	+0.3933*
S100B immediately after operation (μg/L)	+0.5871***
S100B day 1 of operation (μg/L)	+0.5841***
S100B day 5 of operation (μg/L)	+0.4997**

Levels of significance: *P<0.05; **P<0.01; ***P<0.001

Table 3. Serum S100B predicted hospital stay

Dependent variable		β (standard error)	P
Log (log hospital stay (days))			
Independent variable in the model			
Model B1	Constant	+0.7678 (0.2077)	0.0010
	Age (yrs)	+0.0038 (0.0026)	0.1568
	Operation (type)	−0.1611 (0.0627)	0.0161
	Log S100B (day 1 of operation)	+0.1111 (0.0617)	0.0827
	r=0.6912; P=0.0005		
Model B2	Constant	+1.0401(0.0922)	<0.0001
	Operation (type)	−0.1532 (0.0637)	0.0231
	Log S100B (day 1 of operation)	+0.1377 (0.0601)	0.0296
	r=0.6609; P=0.0003		
Model C1	Constant	+0.9558 (0.2116)	0.0001
	Age (yrs)	+0.0032 (0.0023)	0.1843
	Operation (type)	−0.1768 (0.0529)	0.0023
	Log S100B (day 5 of operation)	+0.1378 (0.0547)	0.0178
	r=0.6897; P=0.0004		
Model C2	Constant	+1.1904 (0.1244)	<0.0001
	Operation (type)	−0.1810 (0.0536)	0.0021
	Log S100B (day 5 of operation)	+0.1553 (0.0540)	0.0075
	r=0.6933; P=0.0001		

Log = logarithmic transformation of values

dicted hospital stay, multivariate linear regression analysis was repeated in a stepwise backward manner to account for the influence of the possible confounding variables. As summarized in Table 3, serum S100B predicted the length of hospital stay on day 1 (p=0.0296, model B2) and day 5 (p=0.0075, model C2) independently of the type of operation and age of patients.

Discussion and Conclusions

The results of our study are in good agreement with the results from previous studies using a method of S100B determination with low enough limit of detection[6,12-15]. As in other previously reported studies, we cannot explain why the concentrations of S100B are increased after CABG operation. The increase in S100B concentration probably represents subtle cognitive dysfunction. Using magnetic resonance imaging, cerebral pathology was documented in 30% of patients without clinical signs after CABG[3]. Hardly recognizable brain injury, which could only be identified by hemologic examination, was recorded in 60% of patients after the operation[16].

The possible cause of the increase in S100B concentration is systemic inflammation induced by CPB heart surgery[17]. Systemic inflammation induced by CPB could conceivably increase the blood-brain barrier permeability and increase the bioavailability of S100B. On the other hand, the normal concentration of S100B in cerebrospinal fluid is only 1.7 mg/L, which is considerably less than the increases observed after CPB[6]. It is conceivable that early S100B release occurs through "wash out" from the capillaries when pulsate cerebral blood flow occurs upon return of cardiac action to replace the linear blood flow generated by the roller pump[3].

Differences in protein S100B increase can be influenced by the type of operation, age of patient, operative time, kidney function, use of autotransfusion and use of heparin therapy. All these parameters represent non-brain parameters, which increase S100B serum concentration and do not signify brain tissue damage. According to literature data, their influence should vanish after 48 hours.

Literature data show that the increase in the concentration of S100B is connected with the size of damaged brain tissue[18]. High S100B levels 48 hours after

surgery have a negative predictive value for median term survival. The mortality after the operation was directly connected with the concentration of S100B up to 0.5 mg/L[5].

If S100B is used as a prognostic marker of brain injury after cardiac surgery, 48 hours of the operation would be the earliest time for sample collection to prevent false-positive results. In this way, the non-brain causes of increased S100B values could be abolished. To avoid the influence of non-brain parameters, examinations were done at 24 hours and 5 days of the operation. False-negative results could not be excluded at this time because of the presence of a high variation of S100B results due to different location and size of brain tissue damage. In contrast, in the group of patients with worse prognosis (>0.5 mg/L 48 hours after the operation), the concentrations of S100B were significantly higher just 15 hours of the operation, which could have been ascribed to brain or non-brain sources. We also found the concentrations of S100B on day 1 of the operation to be independent of the type of operation and patient age. Yet, the concentration of S100B was connected with the length of hospital stay, which is an indicator of postoperative complications. On day 5 of the operation, the association between the concentration of S100B and length of hospital stay was even more significant.

Univariant comparison of serum concentration of S100B between patients operated on with or without CABG showed the concentration of S100B immediately after the operation to be higher in the group of patients undergoing CABG. The difference in S100B concentration was lower on day 1 of the operation. On day 5 of the operation, the difference was not noticeable anymore. These results show that the concentration of S100B is particularly dependent on the type of operation, which is in good agreement with literature data on postoperative neuralgic consequences[12-15].

Off-pump surgery causes smaller[6] or no increase[12] in serum S100B concentration. These results suggest that the off-pump operation is less harmful for patients and also causes less postoperative complications. More than 24 hours after the operation, other factors of non brain origin which can cause an increase in S100B concentration are not important anymore, so that the relationship between S100B concentration and the length of hospital stay becomes clearer.

On day 5 of the operation this relationship became even more important because then elevated S100B concentration had a prognostic meaning for postoperative complications. Our data suggest that serum S100B concentration on day 5 of the operation may have a prognostic value in patients without clinical signs of cerebral injury. Of course, it should be emphasized that a study in a larger group of patients is necessary to confirm this observation. Due to a small set of data, statistical calculations used in our pilot trial are only approximate and for now the results have limited statistical significance.

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References

1. FILSOUFI F, RAHMANIAN PB, CASTILLO JG, BRONSTER D, ADAMS DH. Incidence, imaging analysis, and early and late outcomes of stroke after cardiac valve operation. Am J Cardiol 2008;101:1472-8.
2. BABA T, GOTO T, MAEKAWA K, ITO A, YOSHITAKE A, KOSHIJI T. Early neuropsychological dysfunction in elderly high-risk patients after on-pump and off-pump coronary bypass surgery. J Anesth 2007;21:452-8.
3. JONSSON H, JOHNSSON P, ALLING C, WESTABY S, BLOMQUIST S. Significance of serum S100 release after coronary artery bypass grafting. Ann Thorac Surg 1998;65:1639-44.
4. MOTALLEBZADEH R, KANAGASABAY R, BLAND M, KASKI JC, JAHANGIRI M. S100 protein and its relation to cerebral microemboli in on-pump and off-pump coronary artery bypass surgery. Eur J Cardiothorac Surg 2004;25:409-14.
5. JONSSON H, JOHNSSON P, BIRCH-IENSEN M, ALLING C, WESTABY S, BLOMQUIST S. S100B as a predictor of size and outcome of stroke after cardiac surgery. Ann Thorac Surg 2001;71:1433-7.
6. ANDERSON RE, HANSSON LO, VAAGE J. Release of S100B during coronary artery bypass grafting is reduced by off-pump surgery. Ann Thorac Surg 1999;67:1721-5.
7. ZIMMER DB, CORNWALL EH, LANDAR A, SONG W. The S100 protein family: history, function, and expression. Brain Res Bull 1995;37:417-29.
8. FRIEND WC, CLAPOFF S, LANDRY C, BECKER LE, O'HANLON D, ALLORE RJ, *et al*. Cell-specific expression of high levels of human S100 beta in transgenic mouse brain is dependent on gene dosage. J Neurosci 1992;12:4337-46.
9. WHITAKER-AZMITIA PM, WINGATE M, BORELLA A, GERLAI R, RODER J, AZMITIA EC. Transgenic mice overexpressing the neurotrophic factor S-100 beta show neuronal, cytoskeletal and behavioral signs of altered aging processes: implications for Alzheimer's disease and Down's syndrome. Brain Res 1997;776:51-60.

10. HEIZMANN CW, BRAUN K. Changes in Ca(2+)-binding proteins in human neurodegenerative disorders. Trends Neurosci 1992;15:259-64.
11. GAO F, HARRIS DN, SAPSED-BYRNE S, SHARP S. Neurone-specific enolase and Sangtec 100 assays during cardiac surgery: Part I – The effects of heparin, protamine and propofol. Perfusion 1997;12:163-5.
12. WESTABY S, JOHNSSON P, PARRY AJ, BLOMQVIST S, SOLEM JO, ALLING C, *et al*. Serum S100 protein: a potential marker for cerebral events during cardiopulmonary bypass. Ann Thorac Surg 1996;61:88-92.
13. TAGGART DP, MAZEL JW, BHATTACHARYA K, MESTON N, STANDING SJ, KAY JD, *et al*. Comparison of serum S-100 beta levels during CABG and intracardiac operations. Ann Thorac Surg 1997;63:492-6.
14. BLOMQUIST S, JOHNSSON P, LUHRS C, MALMKVIST G, SOLEM JO, ALLING C, *et al*. The appearance of S-100 protein in serum during and immediately after cardiopulmonary bypass surgery: a possible marker for cerebral injury. J Cardiothorac Vasc Anesth 1997;11:699-703.
15. SVENMARKER S, SANDSTROM E, KARLSSON T, JANSSON E, HAGGMARK S, LINDHOLM R, *et al*. Clinical effects of the heparin coated surface in cardiopulmonary bypass. Eur J Cardiothorac Surg 1997;11:957-64.
16. GROCOTT HP, CROUGHWELL ND, AMORY DW, WHITE WD, KIRCHNER JL, NEWMAN MF. Cerebral emboli and serum S100beta during cardiac operations. Ann Thorac Surg 1998;65:1645-9; Discussion 1649-50.
17. MIELCK F, ZIARKOWSKI A, HANEKOP G, ARMSTRONG VW, HILGERS R, WEYLAND A, QUINTEL M, SONNTAG H. Cerebral inflammatory response during and after cardiac surgery. Eur J Anaesthesiol 2005;22:347-52.
18. KORFIAS S, STRANJALIS G, BOVIATSIS E, PSACHOULIA C, JULLIEN G, GREGSON B, MENDELOW AD, SAKAS DE. Serum S-100B protein monitoring in patients with severe traumatic brain injury. Intensive Care Med 2007;33:255-60.

Sažetak

KORELACIJA SERUMSKE KONCENTRACIJE S100B I TRAJANJA HOSPITALIZACIJE U BOLESNIKA PODVRGNUTIH UGRADNJI PREMOSNICE KORONARNE ARTERIJE

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Protein S100B se smatra serumskim biljegom moždanog oštećenja u bolesnika podvrgnutih operacijama srca. U ovom ispitivanju potražili smo odgovor na pitanje korelira li porast serumske koncentracije S100B s duljinom hospitalizacije u bolesnika podvrgnutih ugradnji premosnice koronarne arterije. Serumske koncentracije S100B mjerili smo prije operacije, neposredno nakon operacije, te prvog i petog dana od operacije u 32 bolesnika podvrgnutih ugradnji premosnice koronarne arterije. Medijan (minimum; maksimum) boravka u bolnici bio je 7 (5-34) dana, serumska koncentracija S100B prije operacije 0,075 (0,050; 0,095) μg/L, neposredno nakon operacije 0,840 (0,390; 1,500) μg/L, prvoga dana 0,180 (0,150; 0,280) μg/L i petoga dana nakon operacije 0,100 (0,080; 0,120) μg/L. Nijedan od naših bolesnika nije imao kliničke znakove moždanog oštećenja. Ipak, multivarijantna analiza linearne regresije pokazala je korelaciju serumske koncentracije S100B s trajanjem hospitalizacije prvoga dana (p=0,0296) i petoga dana (p=0,0021) od operacije neovisno o vrsti operacije (uz primjenu kardiopulmonalne obilaznice ili bez nje) i kliničkim značajkama bolesnika. Naši podaci ukazuju na to da bi serumska koncentracija S100B prvoga i petoga dana od operacije mogla imati prognostičku vrijednost u bolesnika bez kliničkih znakova moždanog oštećenja. Ovo naše probno ispitivanje trebalo bi svakako proširiti na veću skupinu bolesnika kako bi se potvrdilo ovo zapažanje.

Ključne riječi: *Premosnica koronarne arterije – štetni učinci; Premosnica koronarne artrije – metode; Proteini B100 – krv; Biološki biljezi – krv*