The diagnostic utility of S-100B protein and TPA in patients with ischemic stroke

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Abstract

OBJECTIVE: The aim of the study is to asses blood plasma concentrations of S-100B protein and Tissue Polypeptide Antigen (TPA) in patients with confirmed ischemic stroke and to correlate these concentrations with stroke severity.

METHODS: S-100B protein and TPA blood plasma concentrations were determined in 47 patients with acute ischemic infarction and in the control population. S-100B protein was assessed on the 1th day, TPA on the 1th, 7th and 14th day. The clinical status was documented using Scandinavian Stroke Scale. The functional deficit after the stroke was scored by Barthel Index.

RESULTS: The analysis of the entire examined group in relation to the control population showed elevated concentrations of S-100B protein (0.47 ng/ml vs. 0.19 g/ml). The highest concentrations were in the severe stroke group (0.89 ng/ml). The assessment of TPA blood plasma concentrations showed higher ones in the examined group of patients: 225.7 U/l on the 1st day; 96.1 U/l on the 7th day; 125.64 U/l on the 14th day after the stroke in relation to the control population.

CONCLUSION: The analysis of obtained results showed significant increase of S-100B protein blood plasma concentrations in patients with severe stroke and TPA in patients with mild stroke. S-100 protein blood plasma concentration assessed on the 1st day after the ischemic stroke is the parameter presenting the highest diagnostic utility and its value above 0.6 ng/ml was obtained only in patients with severe stroke.

ORIGINAL ARTICLE

BACKGROUND

Cardiovascular diseases of the brain are the challenge for the senescent societies. The additional problem for the countries regardless of their development degree is the fact that the stroke concerns more often young people who are professionally active. Therefore we observe the development of neuroimaging techniques such as computed tomography (CT), magnetic resonance imaging (MRI), Doppler ultrasonography. Research whose aim is to find an early and sensitive brain damage marker is still being conducted. The marker should be easy to measure in blood serum and the measurement should be repeatable. Such marker should also have significant prognostic utility as for the course of the stroke. It could even emerge a group of people who are at grater risk of stroke. We put our hopes on putting S-100 protein assays into diagnosis. Assay of soluble fragments of Cytokeratin 18 TPS or TPA could also be useful.

S-100 protein

S-100 protein is a calcium-binding protein synthesized by astroglia cells in all parts of central nervous system. It consists of two subunits – alpha and beta. The protein, especially 100 beta form, is presented in high concentrations in astrocytes and Schwann cells. S-100 protein has two calcium-binding regions – alkaline and acidic. There isn't any presence of this protein in blood serum in physiological conditions, however it is secreted to blood as well as to cerebrospinal fluid in damages of central nervous system. Its presence shows not only the damage of brain cells, but also the damage of blood-brain barrier. Elevate concentration of S-100 protein was observed in other diseases with nervous system damage. These are: trisomy 21, Alzheimer disease, Creutzfeldt-Jakob disease, multiple sclerosis, head injuries. Diagnostic utility of this marker assay was confirmed in neurosurgery for evaluating vessels constriction threat, predicting the return of consciousness in unconscious patients after craniocerebral traumas and also in cardiovascular surgery to assess the condition of nerve tissue during operations performed under cardiopulmonary bypass [1,2,3,4,5,6].

TPA

Cytokeratin 18 is an acidic protein (molecular weight 45 kDa) which is presented in epithelium cells. Tissue Polypeptide Antigen (TPA) is a circulating complex of polypeptide soluble fragments of cytokeratins: 1, 18 and 19. Review of literature shows high utility of TPA assays in neoplasms diagnosis. There are some recent reports about the increase of blood plasma concentrations of TPA in autoimmune diseases, liver cirrhosis [7,8,9,10].

PURPOSE

The aim of the study is to assess blood plasma concentrations of S-100B protein and TPA in patients with confirmed ischemic stroke and to correlate these concentrations with stroke severity. The next aim is to choose the antigen or antigens with particular diagnostic utility in patients with the ischemic stroke.

PATIENTS AND METHODS

47 patients admitted to the neurology ward between year 2000 and 2002 including 27 women and 20 men were enrolled. The mean age of patients was 70.3 years (range 52–89). The cause of admitting these patients was ischemic stroke confirmed by clinical examination and CT of head made on the third day after stroke onset. Patients with known history of disease onset circumstances were included to the examined group. 35 patients were affected with arterial hypertension, 11 were affected with atrial fibrillation and 24 were affected with coronary artery disease. Patients with stroke, central nervous system infection, brain tumor or other neoplasms past over the last 3 months before examination and patients with current acute inflammation or liver diseases were excluded from examination. 10 healthy volunteers suitable for examined group in terms of age and sex were the control population. The study was approved by The Ethical Board of Silesian Medical Academy.

The neurological status of the patients was estimated using Scandinavian Neurological Stroke Scale (SNSS). SNSS is a frequently used scale to estimate the damage extent on admission and short term prognosis in ischemic stroke. On the basis of SNSS patients were divided into two groups: severe stroke group scored 20 points and below and mild stroke group scored 21 and more points. There were 15 patients with quantitative consciousness disorders in severe stroke group and 32 patients without consciousness disorders were in mild stroke group. The estimation was repeated on the 30th day after stroke. Barthel Index, the most frequently used method of functional assessment of the return to every day activity in patients with stroke, was used to asses the degree of return of neurological functions after acute period of the disease. Barthel Index was estimated on the 14th and 30th day after the stroke.

Venous blood samples in quantity of 5 ml were taken from the ulnar vein to dry test tubes on the 1st, 7th and 14th day after stroke and allowed to clot. Then the samples were centrifuged and the assays of protein S-100 in the first day's sample and TPA in all samples were done. Assays of acute-phase protein and iron concentrations, aminotransferases activity in blood serum, blood cells count and erythrocyte sedimentation reaction were done to preclude acute inflammation, iron insufficiency, anemia and liver diseases. TPA and S-100B protein were analyzed using immunoluminometric assays (Byk Sangtec, Sweden).

Statistical analysis

t-Student test was used to compare pairs of arithmetical averages, Wilcoxon's test of order of pairs was used as the non-parametric alternative of t-Student test to asses

the significance of difference between two dependent measurements (dependent variables). Kolmogarow-Smirnow's test was used to assess two measurements with the distribution other than normal. Wilcoxon's test and Kolmogarow-Smirnow's test are commonly used for small groups. The analysis of multiple regression was used to assess the correlation between many independent variables and the dependent variable. Results are showed as arithmetical averages. The significance level was assumed as $p<0.05$.

Table 1. S-100B protein concentrations on the first day after the stroke in all groups of patients and in the control population.

Group name	S-100 protein [ng/ml]	n	p-value
Severe stroke	0.89 ± 0.55	15	<0.001
Mild stroke	0.27 ± 0.15	32	<0.01
Both stroke groups	0.47 ± 0.44	47	<0.001
Control population	0.19 ± 0.03	10	

RESULTS

The examined group of patients and the control population were homogeneous in terms of age which is shown on Figure 1. Results of S-100B protein assays in first day after stroke with taking the division into stroke severity degree into consideration are shown on Figure 2 and in Table 1.

The analysis of the entire examined group in relation to the control population showed elevated concentrations of S-100B protein. The highest concentrations were in the severe stroke group. Lower concentrations, however, still significantly higher than in the control population, were in the mild stroke group.

The assessment of TPA blood plasma concentrations showed higher ones in the examined group of patients: 225.7 U/l on the 1st day ($p<0.025$); 96.1 U/l on the 7th day ($p<0.01$); 125,64 U/l on the 14. day ($p<0.05$) after the stroke in relation to the control population. The analysis taking stroke severity degree into consideration showed that the highest plasma concentration of TPA was assessed in first day after the stroke in the mild stroke group which is presented on Figure 3 and in Table 2.

Correlation tests using the multiple regression methods between both blood plasma markers concentrations and SNSS score on the first day and Barthel on the 14th and 30th day after the stroke showed the highest correlation between S-100B protein concentration and SNSS score in the first day after stroke which is presented on Figure 4. There is statistically significant inverse correlation between S-100B protein concentrations and Barthel Index in 14 and 30 day after stroke resulting from the analysis of linear regression charts. It was additionally observed that the concentration of S-100B protein was lower than 0.6 ng/ml on the day of stroke in patients with good prognosis which is illustrated on Figures 4, 5a and 5b.

DISCUSSION

Last years have brought us an enormous progress in development of techniques which are useful in assessment of neurological status of patients suffering from ischemic stroke. The modern manners of brain examining such as CT, MRI, evoked potentials, Doppler ultrasonography are being developed and are helpful in identification of extent and type of damage focus. These methods are crucial for taking up less or more aggressive treatment. The review of literature shows the increase of researchers interest in the quantitative assay of concentrations of brain damage markers which are released to the blood. Few markers such as neuron-specific enolase (NSE), glial fibrillary acidic protein (GFAP), alkaline myelin protein and S-100 protein have been assessed so far.

Büttner *et al.* [11] found on the basis of their research that in ischemic stroke in the territory of medial cerebral artery (MCA) approximately 80% of patients had the significant S-100 protein blood plasma concentrations increase in at least one blood sample within 10 days after the stroke observation. The greatest increase of concentration concerned patients with massive brain edema and brain midline shift in CT of the head. The continuous increase of S-100 protein concentration showed the bad prognosis as for the survival or disability degree. Guided by these results, we assessed S-100B protein blood plasma

Table 2. TPA blood plasma concentrations in the control population and in examined groups of patients on the 1st, 7th and 14th day after the ischemic stroke.

Group name	TPA [U/l]								
	1st day	p-value	n	7th day	p-value	n	14th day	p-value	n
Severe stroke	40.00 ± 31.1	NS	15	33.90 ± 14.3	NS	10	61.05 ± 50.8	NS	9
Mild stroke	312.75 ± 718.8	<0.01	32	120.0 ± 219.0	<0.005	26	148.90 ± 222.1	<0.005	25
Both stroke groups	225.70 ± 604.2	<0.02	47	96.11 ± 189.3	<0.01	36	125.64 ± 195.1	<0.05	34
Control population	26.88 ± 5.56								10

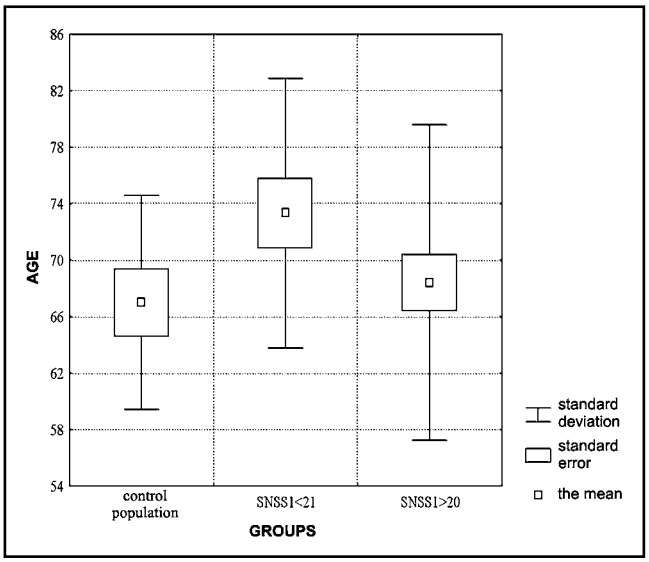

Figure 1. The mean age in the control population and in examined patients groups.

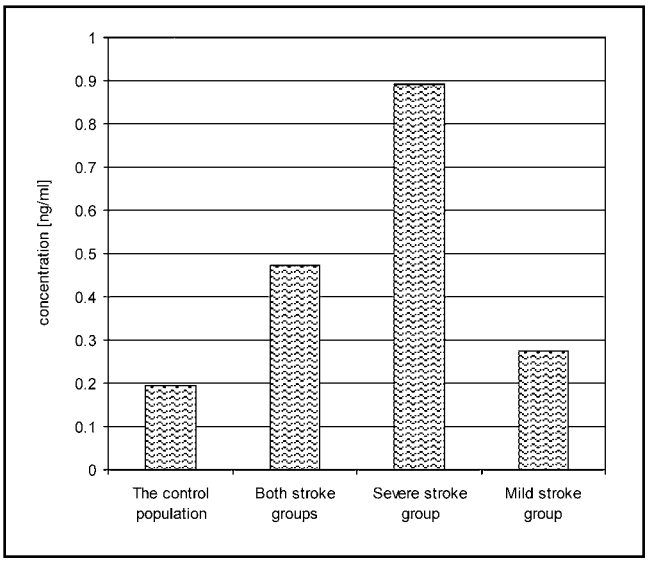

Figure 2. S-100B protein concentrations on the first day after the stroke in all groups of patients and in the control population.

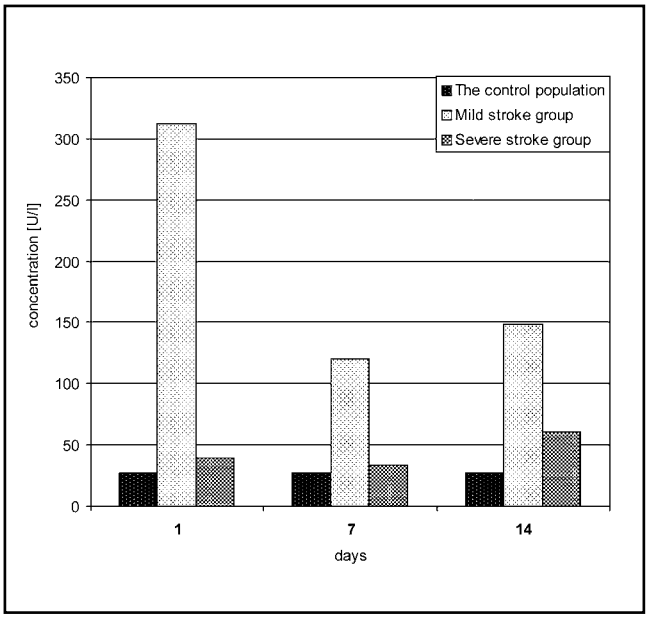

Figure 3. TPA blood plasma concentrations in the control population and in examined groups of patients on the 1st, 7th. and 14th day after the ischemic stroke.

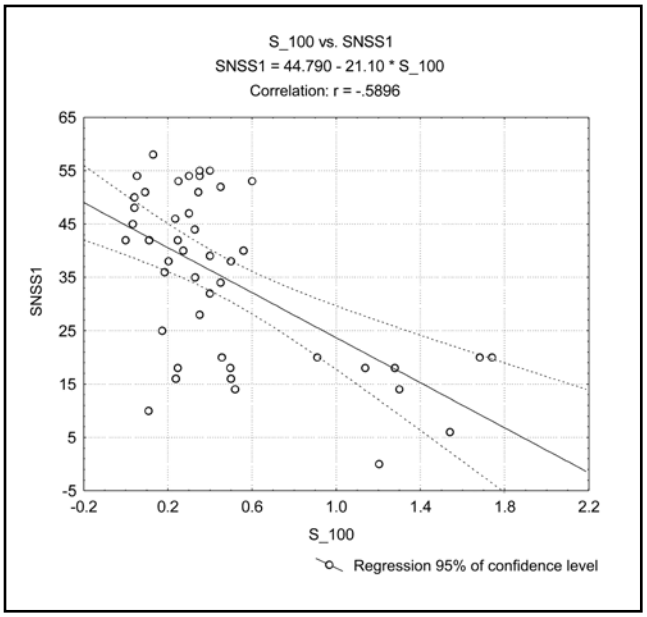

Figure 4. The correlation between S-100B protein blood serum concentrations on the first day after the stroke and SNSS score on the first day after the stroke (SNSS 1) in all examined patients ($p<0.00005$).

concentrations in our own clinical material on the first day after the stroke. The examined patients were divided into two groups with taking the division into stroke severity degree into consideration. Increased concentrations of S-100B protein were observed in all patients with severe stroke and in 15 patients with mild stroke, which is approximately 50%. Concentrations in control population didn't cross the value 0.2 ng/ml which is consistent with the references [12,13,14]. The mean concentration in severe stroke group was at 0.89 ng/ml and statistically significantly differed from the one in mild stroke group and the control population. The detailed statistical analysis enabled us to determine the threshold value of S-100B protein blood plasma concentration above which it is necessary to take an irreversible brain damage into account. The threshold value is at 0.6 ng/ml. The review of literature about utility of S-100 protein assays in different neurological diseases shows the great prognostic

utility of this marker assays. The majority of authors agree that the elevated S-100 protein concentration is the bad prognosis [11,12,15,16,17,18,19].

Our results are similar to those by Martens *et al.* [17]. These authors assessed S-100 protein concentration as the marker of the consciousness return after brain ischemia and recognized the value 0.7 ng/ml as critical. Papers by Missler *et al.* [12] and Büttner *et al.* [11] in which authors confirmed the utility of S-100 protein assays in greater groups of patients have appeared over recent years. They correlate S-100 protein serum concentration with the volume of ischemic focus and neurological condition assessment. The positive relationship with the first parameter and the negative relationship with the second parameter after six months follow-up was proved [11,12]. However, these authors did not correlate S-100 protein concentration either with the status of patients on the first day after the stroke or with the neurological assessment next days. Such correlation was done in our material and it showed a large statistical significance using linear regression methods either comparing to SNSS-1 (in first day after stroke), or with Barthel Index: Barthel-1 (on the 14th day) and Barthel-2 (on the 30th day). Our results are similar to presented by other researchers [12,17,20].

Missler and Büttner [11,12] analyzed S-100 protein concentrations within the first 8 days of the stroke getting the highest concentrations on the 3rd day, however, increased concentrations were affirmed from the day of admission. The delayed increase of S-100 protein concentration is probably the result of the gradual process of glia cells damage and decay. It is a sign of metabolic changes in infarct tissue. Metabolic acidosis leads to cell membrane damage and the water and sodium inflow into the cells directly after ischemia. It is also the cause of cytotoxic edema. S-100 protein concentrations similar to normal within first hours after stroke were stated even in patients with massive brain edema. It seems that only the final damage of nerve tissue involving also the penumbra area causes the increased extracellular concentration of S-100 protein. Recapitulating, the increased concentration of S-100 protein is the result of its releasing from damaged glia cells as a result of ischemia, cytotoxic and angioneurotic edema, and in consequence passing to the systemic fluids through the damaged blood-brain barrier. The constant development of clinical immunodiagnostics and inventing technologies of monoclonal antibodies producing leads to detecting new antigens presented in blood serum in patients suffering from different diseases including neurological. TPS (Tissue Polypeptide Specific Antigen) and TPA are the markers strictly connected with cells proliferation. Antibodies, thanks to which we can affirm TPS and TPA presence, detect in fact the soluble fragments of cytokeratin 8 and 18 or 8, 18 and 19. As we know, the diagnostic utility of TPS and TPA values assays is identical. Antibodies detecting both these markers are aimed first of all at cytokeratin 18, which is strictly connected with cells proliferation [8,21,22].

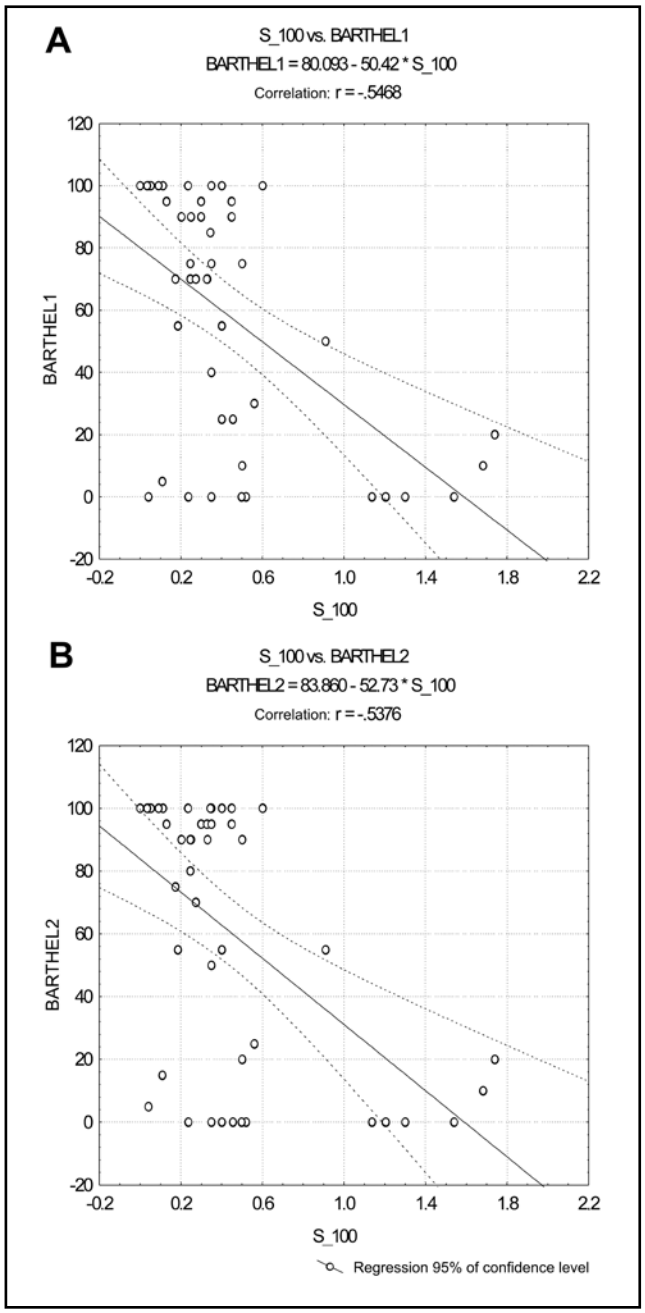

Figure 5. (**A**) The correlation between S-100B protein blood serum concentrations on the first day after the stroke and Barthel Index on the 14th day after the stroke (BARTHEL 1) in all examined patients ($p<0.0001$); (**B**) the correlation between S-100B protein blood serum concentrations on the first day after the stroke and Barthel Index on the 30th day after the stroke (BARTHEL 2) in all examined patients ($p<0.0002$).

Over the last 3 years there have appeared some reports about non-neoplastic increase of TPS and TPA blood plasma concentrations in respiratory system infections, urinary tract infections, autoimmune diseases and liver cirrhosis [9,10]. In the face of increasing interest in cytokeratins role in clinical diagnostics, we made an attempt to asses TPA blood plasma concentration in pa-

tients with ischemic stroke in the paper presented. Serum concentrations of TPA were assessed on the 1st, 7th and 14th day after stroke. The comparison of all patients with ischemic stroke the control population was made and the severity degree was taken into consideration. The highest, statistically significant differences were stated on the first day after the stroke. The mean concentration in all examined patients was 225.7 U/l. The analysis of TPA concentrations taking the division of patients into the stroke severity degrees into consideration is very interesting. High, multiply crossing the norm concentration of TPA was observed on the first day in patients with mild stroke. The mean concentration in this group was 312.75 U/l at high standard deviation. The concentration was over 1 000 U/l in a few patients. The concentrations assayed on the 1st, 7th and 14th day were statistically significant in comparison to the control population as well as between examined groups. The high blood plasma concentration in patients with mild stroke is probably the result of small damage of brain tissues. Probably there is no necrosis in the course of transient ischemia which enables recent proliferation of damage reactive cells like astrocytes or microglia cells. Further observation of these patients showed their fast reconvalescence manifesting in good assessment with neurological scales as well as good biochemical parameters. These patients were discharged within several days in good condition. Unfortunately, we currently can't compare our results to the others because it is probably the first study assessing the diagnostic utility of soluble fragments of cytokeratin 18 in the ischemic stroke. The majority of published papers about TPS and TPA assays are concentrated on their unquestionable utility for detecting the recurrence of neoplasm within non-symptomatic period and for assessing the efficiency of adjuvant treatment.

CONCLUSION

1. The analysis of obtained results showed significant increase of S-100 protein blood plasma concentrations in patients with severe stroke and the increase of TPA blood plasma concentrations in patients with mild stroke.
2. S-100 protein blood plasma concentration assessed on the 1. day after the ischemic stroke is the parameter presenting the highest diagnostic utility and its value above 0.6 ng/ml was obtained only in patients with severe stroke.

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