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Serum level of αII-spectrin breakdown products (SBDPs) as a potential marker of brain ischemia--reperfusion injury after carotid endarterectomy

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Abstract

Introduction: *Stroke remains one of the main causes of morbidity and mortality worldwide. Carotid endarterectomy (CEA) reduces the incidence of ischemic stroke or death in patients with symptomatic carotid artery stenosis more effectively than pharmacological therapy alone. SBDPs (spectrin breakdown products): SBDP120, SBDP145, and SBDP150 are the product of proteolysis of αII-spectrin (280 kDa) — an important structural component of the neuronal cytoskeleton, particularly present in axons. Increased serum level of SBDPs was previously observed in traumatic brain injury, subarachnoid hemorrhage (SAH), or brain ischemia.*

Material and methods: *The aim of our study was to investigate changes in serum levels of SBDP120 and SBDP145 in patients undergoing uncomplicated CEA. The study included 22 patients with severe carotid artery stenosis, qualified for CEA. Blood samples were taken from the antecubital vein at three different intervals (24 h before CEA, 12 and 48 h after surgery). SBDP's serum levels were measured by a commercially available enzyme-linked immunosorbent assay (ELISA).*

Results: *The study showed that serum SBDP120 levels were significantly decreased 48 h after CEA when compared to the level before the surgery. SBDP145 levels were significantly decreased 12 h after the procedure and then remained at a similar level 48 h after CEA.*

Conclusions: *In patients with high-grade carotid artery stenosis SBDP120 and SBDP145 serum level decreases after an uncomplicated CEA, therefore alterations from this curve may be a marker of neurological complications after the procedure. Higher SBDP levels before the procedure may represent brain damage caused by chronic ischemia.*

Keywords: carotid endarterectomy; carotid artery stenosis; stroke; αII-spectrin breakdown products

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Introduction

Stroke remains one of the main causes of morbidity and mortality worldwide, being an important concern of global public health [1]. The definition of a stroke, developed by the World Health Organization (WHO) is a, rapidly developing clinical signs of focal disturbance of cerebral function lasting more than 24 hours with no apparent cause other than of vascular origin" [2]. Ischemic stroke is a more common type than hemorrhagic stroke, and accounts for over 85% of all strokes [3]. The etiology of ischemic stroke (IS) includes atherothrombotic stroke, embolic stroke, cerebral hypoperfusion, and venous thrombosis, with atherothrombotic occlusion of the carotid artery being a major contributor to IS or transient ischemic attack (TIA) [4].

Carotid endarterectomy (CEA) is a surgical procedure, that reduces the incidence of IS or death in patients with severe (≥ 70 to 99% of stenosis), symptomatic (IS, TIA, or retinal TIA) carotid artery stenosis more effective than the pharmacological therapy alone [5–8]. However, this method is not completely free of complications in the perioperative period, and this may include: micro and macro embolism resulting in brain ischemia or ischemia-reperfusion injury and brain edema, caused by clamping and declamping of the internal carotid artery (ICA) during the CEA [9–12].

αII-spectrin (280 kDa) is an important structural component of the neuronal cytoskeleton, particularly present in axons. It plays a crucial role in neuronal integrity. Calpain and caspase-3 are proteolytic enzymes (proteases), which are involved in oncotic necrosis and apoptotic cell death. After injury, αII-spectrin can become a substrate for both enzymes, producing different SBDPs (spectrin breakdown products): SBDP 120, SBDP145, and SBDP150, that have been studied as biomarkers of axonal damage [13–15]. According to previous experimental studies, SBDP 145 and SBDP150 are mainly produced by calpain proteases, and SBDP120 is the product of caspase-3 [14]. SBDPs have been previously found to correlate with the severity of the brain damage after traumatic brain injury (TBI) determined by the GCS score, computed tomography (CT) infarct volume, and 6-month outcome, therefore it may be a potential marker of the severity of brain injury after TBI [16–20].

Spectrin breakdown products were also investigated in patients with aneurysmal subarachnoid hemorrhage (aSAH), relative to development of vasospasm. Serum concentrations of SBDPs were increased before the clinical detection of vasospasm and decreased to baseline after successful treatment [21–24].

In rats, an experimental ischemia, caused by the occlusion of the middle cerebral artery, lead to a strong increase in a level of SBDP120 and SBDP145 six hours after the occlusion in brain tissue and in cerebrospinal fluid (SBDP145), but not in the serum [25].

The advantage of SBDPs as brain damage monitoring biomarkers is the potential to investigate both calpain and caspase-3 activity; SBDP120 is a sensitive marker of caspase-3 activation connected to apoptotic cell death, while SBDP145 and SBDP150 measure a calpain activation associated with oncotic cell death and less with the apoptotic cell death [13, 18].

Material and methods

The patients were admitted to the Department of Vascular Surgery and Angiology of Medical University in Lublin, Poland, and were scheduled to undergo CEA due to internal carotid artery stenosis. The inclusion criteria were: carotid artery stenosis > 50% in symptomatic patients (symptoms of stroke/TIA < 6 months before), or > 60% in asymptomatic patients with at least 1 feature suggesting higher stroke risk on best medical therapy (BMT). The exclusion criteria were: inability to give informed consent, complete occlusion of the internal carotid artery, intracranial artery lesion more significant than the proximal carotid lesion, brain damage in the course of other nervous system diseases, prior ipsilateral CEA, history of disabling stroke (modified Rankin score ≥ 3), active inflammation and expected survival time < 5 years. The study involved 22 patients aged from 57 to 82 with a mean age of 71.36 (standard deviation = 6.51) years. The degree of internal carotid artery stenosis ranged from 70 to 90%. A Neurological examination was performed by a neurologist prior to and after CEA. In this neurological examination, there were no deviations from the normal state in all patients included in the study. Conventional CEA was performed under local anesthesia without the use of a shunt. CEA was performed through a longitudinal arteriotomy, running from the carotid bifurcation to the anterolateral surface of the internal carotid artery (ICA). The carotid artery was clamped, and the arteriotomy was closed with primary sutures. No postsurgical complications were observed. Demographic information and pertinent medical history of the patients are summarized in Table 1.

The degree of internal carotid artery stenosis was determined based on a high-resolution USG Doppler examination, performed with a Toshiba Aplio 500 device with a high-frequency (11 MHz) linear probe. The sonographer was a vascular medicine specialist who was unaware of the subject's clinical state.

Table 1. Characteristics of patients

Patient ID	Sex	Age	Location	%	Stroke/TIA	Symptoms	Other diseases
1	M	74	R	90	No	Tinnitus, hypoacusis	None
2	F	68	L	90	No	Tinnitus, dizziness	Diabetes, arterial hypertension, ischemic heart disease
3	M	57	R	70	No	None	Ischemic heart disease
4	M	78	R	80	No	Visual disturbances	Diabetes, arterial hypertension
5	M	74	L	90	No	Tinnitus, dizziness	Diabetes, arterial hypertension, ischemic heart disease
6	F	67	R	90	Stroke	Dizziness	Diabetes, arterial hypertension
7	M	67	L	90	Stroke	Hemiparesis	Diabetes, arterial hypertension
8	F	79	L	80	No	None	Arterial hypertension
9	M	78	R	90	Stroke	Hemiparesis	Arterial hypertension
10	F	63	L	90	No	Tremor	Diabetes, arterial hypertension
11	M	63	L	80	No	None	Arterial hypertension, ischemic heart disease
12	M	74	R	90	Stroke	None	Arterial hypertension
13	M	63	L	90	Stroke	Hemiparesis	Arterial hypertension
14	F	82	L	80	No	Dizziness	Arterial hypertension
15	M	74	L	90	No	None	Diabetes, arterial hypertension, ischemic heart disease
16	M	76	L	80	Stroke	None	Arterial hypertension
17	M	76	L	85	TIA	None	Diabetes, arterial hypertension, ischemic heart disease
18	F	64	L	90	TIA	None	Diabetes, arterial hypertension
19	F	72	L	70	No	None	Arterial hypertension
20	M	77	L	90	Stroke	Hemiparesis	Arterial hypertension
21	M	67	R	90	Stroke	Hemiparesis	Diabetes, arterial hypertension
22	M	77	L	80	No	Dizziness, visual disturbances	Arterial hypertension

ID — identification, M — male, F — female, TIA — transient ischemic attack, L — left, R — right

Based on Doppler studies, patients were qualified for the CEA procedure as determined by the guidelines set forth by the European Society of Vascular Surgery. Patients with severe carotid artery stenosis were identified using criteria established by NASCET (North American Symptomatic Carotid Endarterectomy Trial) according to the following formula: % ICA stenosis = (1-[narrowest ICA diameter/diameter normal distal cervical ICA]) × 100 [8].

Serum samples were taken from the antecubital vein of patients at three different times: within 24 hours preoperatively to CEA, 12 h postoperatively, and 48 hours postoperatively.

Serum for specific protein analysis was obtained by centrifugation of whole blood at 3000 rpm (603 × g) for 15 min in a laboratory centrifuge (MPW-350R; MPW Medical Instruments, Warsaw, Poland) at a temperature of 4°C and stored in –80°C prior analyses. Plasma without signs of hemolysis was analyzed using a quantitative sandwich enzyme-linked immunosorbent assay (ELISA) technique. The protocols were adapted from a commercially developed assay manufactured by Bioassay Technology Laboratory (BT Lab, Zhejiang, China). The concentrations of human alpha II-spectrin breakdown products: SBDP 120 (Cat. No: E3791Hu) and SBDP145 (Cat. No: E4005Hu) were quantified based on the optical density (OD) at 450 nm using the BioTek ELx808™ Absorbance Microplate Reader (BioTek, Winooski, VT, USA). Samples for each participant were diluted to fit the range of the standard curve and run in duplicate on the same plate. Briefly, the plates have been pre-coated with a human antibody, specific for each analyzed protein. A specific biotinylated antibody was added to sample each well. Then, streptavidin-HRP was added to the sample and standard wells. After incubation, the plates were washed with washing buffer

Table 2. Serum levels of SBDP120 and a comparative analysis

	N	SBDP 120 level [ng/ml]				p
		Mean	Median	SD	SE	
Before	20	122.138	125.696	53.402	11.941	p = 0.0267
12 h after	22	114.720	116.150	51.367	10.951	
48 h after	22	91.812	85.156	39.980	8.523	
Difference				Significance		
Before — 12h after				p = 0.3212		
Before — 48h after				p = 0.0146*		
12h after — 48h after				p = 0.0546		

SE — standard error; SD — standard deviation, N — number of patients; *statistically significant

5× with an automatic plate washer. Substrate solutions were added and once again the plates were incubated. The reaction was terminated by the addition of a stop solution. The concentration of protein levels in samples was calculated based on the standard curves using the average of the duplicate values.

The distribution of the collected data was evaluated using the Shapiro-Wilk's test, showing normal distribution for SBDP120, and not normal for SBDP145. Furthermore, data on the SBDP120 levels were analyzed using one-way repeated measures ANOVA test with post hoc t-test using Bonferroni correction. For the SBDP145 non-parametric methods: one-way repeated measures ANOVA Friedman, and Wilcoxon tests were used to determine differences between the variables.

Correlation analysis was performed using the Spearman rank correlation.

The SBDP120 and SBDP145 values were expressed in ng/ml, and. The values of $p < 0.05$ were considered significant.

The study was approved by the Ethics Committee of the Lublin Medical University (KE-0254/82/2021) (29.04.2021).

Results

The repeated measures ANOVA test showed that a sampling time significantly ($p < 0.05$) affected serum SBDP120 levels; $F(2, 38) = 3.99$, $p = 0.027$. Serum SBDP120 concentrations in patients and a comparative analysis are presented in Table 2.

The post-hoc paired t-test using a Bonferroni corrected $\alpha = 0.0166$ (0.05/3) indicated that the SBDP120 level was statistically significantly decreased 48 h after CEA as compared with the levels measured prior to surgery ($p < 0.0166$, Fig. 1). However, the difference in serum SBDP120 levels prior to surgery 12 h after CEA, and 12 h after CEA compared to 48 h

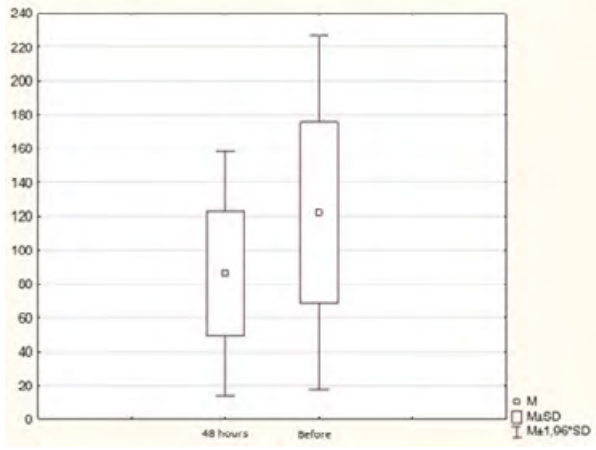

Figure 1. SBDP120 levels in patients before and 48 h after the procedure, Bonferroni $p = 0.0146$* (M — mean; SD — standard deviation); *statistically significant

after the procedure was not statistically significant ($p > 0.0166$).

There was no difference in serum SBDP-120 concentrations in three measurements between males and females ($p > 0.05$). The difference in serum SBDP-120 concentrations between younger (<69 years) and older (> 69 years) patients in the three measurements was not significant ($p > 0.05$).

SBDP145 serum concentration also decreased 12 h after the CEA surgery compared to the preoperative period, and then after 48 h did not significantly change compared to the level 12 h after the procedure. Serum SBDP145 concentrations in patients and a comparative analysis are presented in Table 3.

The repeated measures ANOVA test showed that a sampling time significantly ($p < 0.05$) affected serum SBDP145 levels; $p = 0.00783$.

The post-hoc paired Wilcoxon test indicated that the SBDP145 level was statistically significantly decreased 12 h after CEA as compared with the levels measured prior to surgery ($p < 0.0072$, Fig. 2). However,

Table 3. Serum levels of SBDP145 and a comparative analysis.

	N	SBDP 145 level [ng/ml]				p
		Mean	Median	SD	SE	
Before	21	9.110	7.370	4.009	0.875	p = 0.0078
12 h after	21	8.451	6.770	4.673	1.019	
48 h after	22	8.444	7.665	3.882	0.828	
Difference				Significance		
Before — 12h after				p = 0.00719*		
Before — 48h after				p = 0.130546		
12h after — 48h after				p = 0.614272		

SE — standard error; SD — standard deviation, N — number of patients; *statistically significant

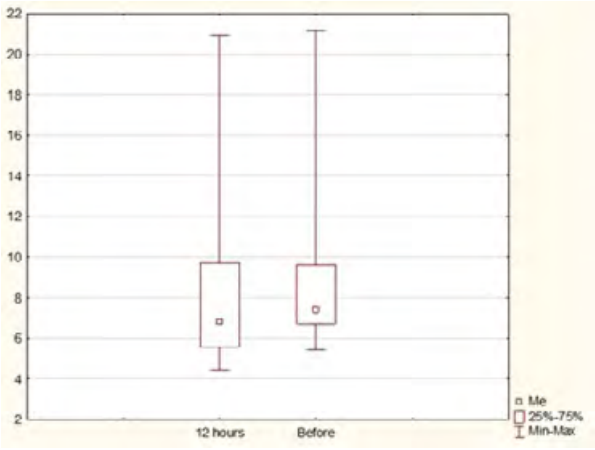

Figure 2. SBDP145 levels in patients before and 12 h after the procedure, p = 0.0146* (Me — median); *statistically significant

the difference in serum SBDP145 levels prior to surgery 48 h after CEA (p = 0.130546), and 48 h after CEA compared to 12 h after the procedure (p = 0.614272) was not statistically significant.

Moreover, there was no difference in serum SBDP-145 concentrations in three measurements between males and females (p > 0.05). The difference in serum SBDP-145 concentrations between younger (< 69 years) and older (> 69 years) patients in three measurements was not significant (p > 0.05).

Discussion

αII-spectrin breakdown products are the molecules, that were previously investigated as potential biomarkers of brain damage in numerous neurological pathologies such as traumatic brain injury (TBI), subarachnoid hemorrhage and ischemic stroke (in rats), or in Alzheimer's disease. However, up to now, it has not been studied in a perioperative period of carotid endarterectomy. Therefore, our study is the first to reveal, that SBDP120 and SBDP145 serum levels decrease after an uncomplicated CEA.

Preclinical studies, on the animal rat model, conducted by Pike et al. (2004), and Ringger et al. (2002) provided the first evidence, that SBDPs can be detected in CSF after ischemic-reperfusion brain injury and traumatic brain injury, and therefore can be used as biochemical markers in a rodent model of transient focal stroke in rats [26, 27].

Farkas et al. (2005), investigated a group of 12 TBI patients with raised intercranial pressure and found that SBDP levels were elevated, compared to the controls, concluding they can be useful in the clinical monitoring of patients with TBI [28].

Early in 2006, Cardali et al. held a prospective, case-controlled study, including 8 patients with severe TBI in which SBDP levels were measured in a CSF at 6, 12, 24, 48, 72, and 96 h following the injury. Authors found, that in TBI patients SBDP levels were significantly increased compared to control patients at all time points examined and that in patients with worse outcomes SBDP's levels remained elevated or failed to decline. The study provided the first evidence, that SBDPs could be a reliable marker of severe TBI in humans [29].

Later, Pineda et al. (2007), examined levels of SBDPs in cerebrospinal fluid (CSF) from adults with severe TBI and the relationship between these levels, severity of injury, and clinical outcome in a group of 41 patients with severe TBI, defined by a Glasgow Coma Scale (GCS) score of < or = 8. SBDP level was measured at 6, 12, 24, 48, 72, 96, and 120 h following TBI and analyzed for SBDPs. The authors concluded, that SBDP levels in CSF were significantly increased in TBI patients at several time points after injury, compared to control subjects. The time course of calpain-mediated SBDP150 and SBDP145 differed from that of caspase-3-mediated SBDP120 during the post-injury period examined. Mean SBDP values measured early after injury correlated with the severity of injury, computed tomography (CT) scan findings, and outcome at 6 months post-injury [30].

Brophy et al (2009), investigated a group of 38 patients with severe TBI, finding a positive correlation between patients with longer elevations in intercranial pressure, and the level of SBDP120, SBDP 145, and SBDP150, and was the first attempt to describe CSF exposure and kinetic characteristics of SBDPs after severe TBI. Authors observed evidence of a greater production of calpain-mediated biomarkers (SBDP145, SBDP150), than caspase–3–mediated biomarkers (SBDP120) in the acute phase following severe TBI [18].

Mondello et al (2010) found that levels of SBDP120 were significantly higher in older patients than in younger patients, suggesting increased apoptotic processes in elderly people. Moreover, the study showed that CSF SBDP levels can predict injury severity and mortality after severe TBI, and can be useful complements to clinical assessment [31].

After that, a couple of other studies confirmed the diagnostic and prognostic utility of SBDPs in TBI [20, 32–34].

In 2023 a meta-analysis held by Liu et al, including 10 studies and 417 participants, confirmed, that SBDPs can be useful biomarkers for the diagnosis and prognosis of TBI [35].

SBDPs were also investigated, as a potential marker of aneurysmal subarachnoid hemorrhage (aSAH), in a group of 40 patients (20 with aSAH, and 20 controls), by Papa et al. (2018). The study revealed that SBDPs could serve as an indicator of aSAH, and are associated with the severity of hemorrhage and early mortality [24].

Similar results were obtained by Lewis et al (2007), who concluded that SBDP levels were significantly increased in patients with aSAH, and increased significantly over baseline level up to 12 hours before the onset of cerebral arterial vasospasm. Moreover, differential expression of SBDPs suggests oncotic necrotic proteolysis may be predominant in acute brain injury after aSAH and cerebral arterial vasospasm [36].

SBDPs have also been investigated as potential indicators of brain damage caused by ischemia in the rat model in some preclinical studies. Zhang et al. [2002], found that the activity of calpains and caspases in the adult rat brain following 10 min of transient forebrain ischemia is increased. Western blots of cortical, striatal, and hippocampal homogenates demonstrated an alpha-spectrin cleavage pattern indicative of predominant calpain activity, with a peak between 24 and 48 h after reperfusion [37]. Pike et al (2004), examined the accumulation of SBDPs in CSF of rodents subjected to 2 hours of transient focal cerebral ischemia produced by occlusion of the middle cerebral artery followed by reperfusion. The study showed that levels of SBDPs (especially SBDP120) were increased in CSF after injury, while were undetectable in the control group [26]. Ren et al (2013), investigated SBDP level in rats with ischemic and hemorrhagic strokes. αII-spectrin breakdown products (SBDP150, SBDP145) were strongly increased after 6h after induced ischemia while remaining on the normal level in hemorrhagic stroke [25].

Spectrin breakdown products were not previously investigated in the perioperative period of carotid endarterectomy. However, there were some other molecules, considered as the brain damage markers, that were previously studied before and after this procedure.

The molecules that have been best recognized under these circumstances are neuron-specific enolase (NSE) and S-100B protein.

Connolly ES Jr et al. (2001) investigated a group of 25 patients who underwent CEA, divided into: injured (those who exhibited significant declines in neuropsychometric test performance), and uninjured patients, finding that injured patients presented significantly higher S100B levels, compared with uninjured patients, at 24, 48, and 72 hours after surgery. There were no significant differences in neuron-specific enolase levels for injured and uninjured patients at any time point [38].

Brightwell et al. investigated NSE and S100B levels in a group of 52 patients with carotid artery stenosis, of whom 28 underwent CEA and 24 carotid artery stenting (CAS). Baseline levels of NSE and S100B were significantly higher compared to the normal population. S-100β increased significantly at 24 hours in patients with a post-operative neurological deficit ($p = 0.015$) and in those with emboli detected by the perioperative trans-cranial Doppler examination. Also, a non-significant trend of transient rise of S-100β levels in the CAS group was observed, while levels in the CEA group appear unchanged. NSE appeared to rise at 48 hours post-operatively in the CEA group and decline in the CAS group, but these differences were also not statistically significant [39].

Rasmussen et al. (2006) measured serum levels of neuron-specific enolase (NSE) and S100B in a group of 22 patients undergoing carotid endarterectomy, pre- and postoperatively (12, 24, 36 and 48 h. after the CEA). In the study group, NSE level was significantly higher before carotid artery surgery and decreased postoperatively, while S100B level did not significantly change. No correlation between the change in cognitive function and the changes in blood levels of either NSE or S-100β protein was found [40]. These results were similar to those obtained in our study, where serum levels of SBDP120 and SBDP145 decreased after the CEA.

The other study, held by Mussack et al on a group of 46 patients with carotid artery stenosis, treated with CEA or CAS, investigated serum levels of S-100B pro-

tein in the perioperative period of CEA and CAS. The authors found that CEA but not CAS caused a transient increase in the S-100B serum levels, which later returned to baseline levels. In addition, prolonged elevation of S-100B serum levels was associated with the development of postoperative neurological deficits [41].

NSE and S100-B were not the only markers, that were studied in the perioperative period of CEA.

Terlecki et al. (2014) held a study on a group of 40 patients undergoing CEA or CAS, investigating perioperative plasma levels of kynurenic acid (KYNA). Baseline plasma KYNA concentrations before the surgery were higher in patients with unstable carotid plaque undergoing CEA than in patients with stable carotid plaque undergoing CEA and patients undergoing CAS. Moreover, KYNA concentration increased during the postoperative period in all studied groups. Higher plasma KYNA concentrations were observed in patients with postoperative neurological disorders [42].

Later, Ilzecki et al. investigated a group of molecules — potential biomarkers of brain damage — in the perioperative period of uncomplicated CEA, in the group of 25 patients. Serum Carnosine Dipeptidase 1 (CNDP1), Ubiquitin C — Terminal Hydrolase L1 (UCHL1), Microtubule-associated protein tau (MAPt) and myelin basic protein (MBP) levels were significantly decreased 12 h after CEA when compared to the level before the surgery, and then normalized 48 h after CEA [43, 44]. Three other molecules: glial fibrillary acidic protein (GFAP), neurofilament light polypeptide (NEFL), and brain lipid-binding protein (FABP7) levels did not statistically significantly change in the perioperative period of CEA [45, 46]. In contrast, the serum level of NSE was statistically significantly increased 48 h after CEA as compared with the levels measured 12 h after surgery and before surgery ($p < 0.05$) [47].

In our study, the level of SBDP120 and SBDP145 statistically significantly decreased after uncomplicated CEA, but the molecules showed different time curves, with SBDP 120 level decreasing gradually with the lowest level 48 hours after CEA, while SBDP145 decreased after 12 hours, and then stayed at the same level 48 hours after CEA. This difference may be explained by the different pathophysiological pathways for both of the molecules, where SBDP 145 is mainly produced by calpain proteases, and SBDP120 is the product of caspase-3.

The results obtained in our study are similar to some of the previous research, in which the serum level of brain damage markers (CNDP1, UCHL1, MAPt, MBP) significantly decreased in uninjured patients, after the uncomplicated CEA. The reason for that may be a normalization of perfusion after an uncomplicated CEA. Higher baseline levels of brain damage markers such as SBDP120 and SBDP145 could be a result of chronic brain ischemia caused by a high-grade internal carotid artery stenosis which could be responsible for the chronic damage of the brain. Therefore, it is reasonable, that when the perfusion normalizes, SBDP's levels fall with time. Similar results obtained by Rasmussem and Brightwell who investigated NSE levels before and after uncomplicated CEA, and Ilzecki, who investigated levels of CNDP1, UCHL1, MAPt, and MBP seem to support this hypothesis. On the other hand, some other brain damage markers levels, like S100B and KYNA appeared to rise after CEA, which may be a result of their higher sensitivity in detecting silent brain ischemia after the CEA, caused by clamping and declamping of the artery, microemboli, ischemia-reperfusion, etc.

The study was conducted on a group of 22 patients, which can be considered as one of the limitations of this research. The other could be a lack of a control group of healthy subjects, or a group of patients with neurological complications after the CEA, however, the aim of the study was to show how SBDPs react in patients undergoing uncomplicated CEA. The studies including patients with neurological complications could be a matter of study in the future.

Conclusions

Higher levels of SBDP and SBDP145 before the CEA may represent brain damage caused by chronic ischemia, which is reduced by successful, uncomplicated CEA.

SBDP 120 and SBDP145 serum levels decrease after an uncomplicated CEA in patients with high-grade carotid artery stenosis therefore alterations from this curve may be a marker of neurological complications after the procedure.

SBDPs may not be enough sensitive markers to detect silent ischemia, caused by CEA (clamping and declamping of the artery, microemboli, ischemia--reperfusion injury).

Up to now, our study is the first to investigate the influence of the uncomplicated CEA on the level of SBDPs, and it presents the data on a characteristic time curve of this molecule in the perioperative period of CEA.

Due to the limitations of our study, there is a need for further investigations on a bigger group of patients, including patients with neurological complications after the CEA, and a control group of healthy subjects.

The knowledge of how different brain damage marker levels are affected by CEA, may in the future lead to the development of a diagnostic panel, useful in detecting neurological complications after the procedure.

Conflict of interest

None.

References

1. Favate AS, Younger DS. Epidemiology of ischemic stroke. Neurol Clin. 2016; 34(4): 967–980, doi: 10.1016/j.ncl.2016.06.013, indexed in Pubmed: 27720004.
2. The World Health Organization MONICA Project (monitoring trends and determinants in cardiovascular disease): a major international collaboration. WHO MONICA Project Principal Investigators. J Clin Epidemiol. 1988; 41(2): 105–114, doi: 10.1016/0895-4356(88)90084-4, indexed in Pubmed: 3335877.
3. Deb P, Sharma S, Hassan KM. Pathophysiologic mechanisms of acute ischemic stroke: An overview with emphasis on therapeutic significance beyond thrombolysis. Pathophysiology. 2010; 17(3): 197–218, doi: 10.1016/j.pathophys.2009.12.001, indexed in Pubmed: 20074922.
4. Yip HK, Sung PH, Wu CJ, et al. Carotid stenting and endarterectomy. Int J Cardiol. 2016; 214: 166–174, doi: 10.1016/j.ijcard.2016.03.172, indexed in Pubmed: 27061654.
5. Randomised trial of endarterectomy for recently symptomatic carotid stenosis: final results of the MRC European Carotid Surgery Trial (ECST). The Lancet. 1998; 351(9113): 1379–1387, doi: 10.1016/s0140-6736(97)09292-1.
6. Mayberg MR. Carotid endarterectomy and prevention of cerebral ischemia in symptomatic carotid stenosis. Veterans Affairs Cooperative Studies Program 309 Trialist Group. JAMA: The Journal of the American Medical Association. 1991; 266(23): 3289–3294, doi: 10.1001/jama.266.23.3289.
7. Barnett HJ, Taylor DW, Eliasziw M, et al. Benefit of carotid endarterectomy in patients with symptomatic moderate or severe stenosis. North American Symptomatic Carotid Endarterectomy Trial Collaborators. N Engl J Med. 1998; 339(20): 1415–1425, doi: 10.1056/NEJM199811123392002, indexed in Pubmed: 9811916.
8. Ferguson GG, Eliasziw M, Barr HW, et al. The North American Symptomatic Carotid Endarterectomy Trial: surgical results in 1415 patients. Stroke. 1999; 30(9): 1751–1758, doi: 10.1161/01.str.30.9.1751, indexed in Pubmed: 10471419.
9. Gupta N, Corriere MA, Dodson TF, et al. The incidence of microemboli to the brain is less with endarterectomy than with percutaneous revascularization with distal filters or flow reversal. J Vasc Surg. 2011; 53(2): 316–322, doi: 10.1016/j.jvs.2010.08.063, indexed in Pubmed: 21129899.
10. Backhaus R, Boy S, Fuchs K, et al. Hyperperfusion syndrome after MCA embolectomy - a rare complication? Am J Case Rep. 2013; 14: 513–517, doi: 10.12659/AJCR.889672, indexed in Pubmed: 24340127.
11. Lieb M, Shah U, Hines G. Cerebral hyperperfusion syndrome after carotid intervention. cardiology in review. 2012; 20(2): 84–89, doi: 10.1097/crd.0b013e318237eef8, indexed in Pubmed: 22183061.
12. Capoccia L, Sbarigia E, Rizzo A, et al. Contralateral occlusion increases the risk of neurological complications associated with carotid endarterectomy. Int J Vasc Med. 2015; 2015: 1–8, doi: 10.1155/2015/942146, indexed in Pubmed: 25705519.
13. Dash PK, Zhao J, Hergenroeder G, et al. Biomarkers for the diagnosis, prognosis, and evaluation of treatment efficacy for traumatic brain injury. Neurotherapeutics. 2010; 7(1): 100–114, doi: 10.1016/j.nurt.2009.10.019, indexed in Pubmed: 20129502.
14. Zhang Z, Larner SF, Liu MC, et al. Multiple alphaII-spectrin breakdown products distinguish calpain and caspase dominated necrotic and apoptotic cell death pathways. Apoptosis. 2009; 14(11): 1289–1298, doi: 10.1007/s10495-009-0405-z, indexed in Pubmed: 19771521.
15. Glushakova OY, Glushakov AV, Miller ER, et al. Biomarkers for acute diagnosis and management of stroke in neurointensive care units. Brain Circ. 2016; 2(1): 28–47, doi: 10.4103/2394-8108.178546, indexed in Pubmed: 30276272.
16. Pineda JA, Lewis SB, Valadka AB, et al. Clinical significance of alphaII-spectrin breakdown products in cerebrospinal fluid after severe traumatic brain injury. J Neurotrauma. 2007; 24(2): 354–366, doi: 10.1089/neu.2006.003789, indexed in Pubmed: 17375999.
17. Siman R, Toraskar N, Dang A, et al. A panel of neuron-enriched proteins as markers for traumatic brain injury in humans. J Neurotrauma. 2009; 26(11): 1867–1877, doi: 10.1089/neu.2009.0882, indexed in Pubmed: 19811094.
18. Brophy GM, Pineda JA, Papa L, et al. alphaII-Spectrin breakdown product cerebrospinal fluid exposure metrics suggest differences in cellular injury mechanisms after severe traumatic brain injury. J Neurotrauma. 2009; 26(4): 471–479, doi: 10.1089/neu.2008.0657, indexed in Pubmed: 19206997.
19. Czeiter E, Mondello S, Kovacs N, et al. Brain injury biomarkers may improve the predictive power of the IMPACT outcome calculator. J Neurotrauma. 2012; 29(9): 1770–1778, doi: 10.1089/neu.2011.2127, indexed in Pubmed: 22435839.
20. Papa L, Robertson CS, Wang KKW, et al. Biomarkers improve clinical outcome predictors of mortality following non-penetrating severe traumatic brain injury. Neurocrit Care. 2015; 22(1): 52–64, doi: 10.1007/s12028-014-0028-2, indexed in Pubmed: 25052159.
21. Lad SP, Hegen H, Gupta G, et al. Proteomic biomarker discovery in cerebrospinal fluid for cerebral vasospasm following subarachnoid hemorrhage. J Stroke Cerebrovasc Dis. 2012; 21(1): 30–41, doi: 10.1016/j.jstrokecerebrovasdis.2010.04.004, indexed in Pubmed: 20851633.
22. Lewis SB, Velat GJ, Miralia L, et al. Alpha-II spectrin breakdown products in aneurysmal subarachnoid hemorrhage: a novel biomarker of proteolytic injury. J Neurosurg. 2007; 107(4): 792–796, doi: 10.3171/JNS-07/10/0792, indexed in Pubmed: 17937225.
23. Siman R, Giovannone N, Toraskar N, et al. Evidence that a panel of neurodegeneration biomarkers predicts vasospasm, infarction, and outcome in aneurysmal subarachnoid hemorrhage. PLoS One. 2011; 6(12): e28938, doi: 10.1371/journal.pone.0028938, indexed in Pubmed: 22174930.
24. Papa L, Rosenthal K, Silvestri F, et al. Evaluation of alpha-II--spectrin breakdown products as potential biomarkers for early recognition and severity of aneurysmal subarachnoid hemorrhage. Sci Rep. 2018; 8(1): 13308, doi: 10.1038/s41598-018-31631-y, indexed in Pubmed: 30190542.
25. Ren C, Zoltewicz S, Guingab-Cagmat J, et al. Different expression of ubiquitin C-terminal hydrolase-L1 and αII-spectrin

in ischemic and hemorrhagic stroke: Potential biomarkers in diagnosis. Brain Res. 2013; 1540: 84–91, doi: 10.1016/j.brainres.2013.09.051, indexed in Pubmed: 24140110.
26. Pike BR, Flint J, Dave JR, et al. Accumulation of calpain and caspase-3 proteolytic fragments of brain-derived alphaII--spectrin in cerebral spinal fluid after middle cerebral artery occlusion in rats. J Cereb Blood Flow Metab. 2004; 24(1): 98–106, doi: 10.1097/01.WCB.0000098520.11962.37, indexed in Pubmed: 14688621.
27. Ringger NC, O'Steen BE, Brabham JG, et al. A novel marker for traumatic brain injury: CSF alphaII-spectrin breakdown product levels. J Neurotrauma. 2004; 21(10): 1443–1456, doi: 10.1089/neu.2004.21.1443, indexed in Pubmed: 15672634.
28. Farkas O, Polgár B, Szekeres-Barthó J, et al. Spectrin breakdown products in the cerebrospinal fluid in severe head injury--preliminary observations. Acta Neurochir (Wien). 2005; 147(8): 855–861, doi: 10.1007/s00701-005-0559-6, indexed in Pubmed: 15924207.
29. Cardali S, Maugeri R. Detection of alphaII-spectrin and breakdown products in humans after severe traumatic brain injury. J Neurosurg Sci. 2006 Jun;50(2):25-31. , indexed in Pubmed: 16841024.
30. Pineda JA, Lewis SB, Valadka AB, et al. Clinical significance of alphaII-spectrin breakdown products in cerebrospinal fluid after severe traumatic brain injury. J Neurotrauma. 2007; 24(2): 354–366, doi: 10.1089/neu.2006.003789, indexed in Pubmed: 17375999.
31. Mondello S, Robicsek SA, Gabrielli A, et al. αII-spectrin breakdown products (SBDPs): diagnosis and outcome in severe traumatic brain injury patients. J Neurotrauma. 2010; 27(7): 1203–1213, doi: 10.1089/neu.2010.1278, indexed in Pubmed: 20408766.
32. Chen S, Shi Q, Zheng S, et al. Role of α-II-spectrin breakdown products in the prediction of the severity and clinical outcome of acute traumatic brain injury. Exp Ther Med. 2016; 11(5): 2049–2053, doi: 10.3892/etm.2016.3153, indexed in Pubmed: 27168849.
33. Frankel M, Fan L, Yeatts SD, et al. Association of very early serum levels of S100B, glial fibrillary acidic protein, ubiquitin C-terminal hydrolase-L1, and spectrin breakdown product with outcome in ProTECT III. J Neurotrauma. 2019; 36(20): 2863–2871, doi: 10.1089/neu.2018.5809, indexed in Pubmed: 30794101.
34. Berger RP, Hayes RL, Richichi R, et al. Serum concentrations of ubiquitin C-terminal hydrolase-L1 and αII-spectrin breakdown product 145 kDa correlate with outcome after pediatric TBI. J Neurotrauma. 2012; 29(1): 162–167, doi: 10.1089/neu.2011.1989, indexed in Pubmed: 22022780.
35. Liu Y, Yao X, Lv X, et al. The role of spectrin breakdown products in patients with traumatic brain injury: a systematic review and meta-analysis. Neurol Sci. 2023; 44(4): 1171–1183, doi: 10.1007/s10072-022-06558-y, indexed in Pubmed: 36547778.
36. Lewis SB, Velat GJ, Miralia L, et al. Alpha-II spectrin breakdown products in aneurysmal subarachnoid hemorrhage: a novel biomarker of proteolytic injury. J Neurosurg. 2007; 107(4): 792–796, doi: 10.3171/JNS-07/10/0792, indexed in Pubmed: 17937225.
37. Zhang C, Siman R, Xu YA, et al. Comparison of calpain and caspase activities in the adult rat brain after transient forebrain ischemia. Neurobiol Dis. 2002; 10(3): 289–205, doi: 10.1006/nbdi.2002.0526, indexed in Pubmed: 12270691.
38. Connolly ES, Winfree CJ, Rampersad A, et al. Serum S100B protein levels are correlated with subclinical neurocognitive declines after carotid endarterectomy. Neurosurgery. 2001; 49(5): 1076–82; discussion 1082, doi: 10.1097/00006123-200111000-00010, indexed in Pubmed: 11846900.
39. Brightwell RE, Sherwood RA, Athanasiou T, et al. The neurological morbidity of carotid revascularisation: using markers of cellular brain injury to compare CEA and CAS. Eur J Vasc Endovasc Surg. 2007; 34(5): 552–560, doi: 10.1016/j.ejvs.2007.06.016, indexed in Pubmed: 17719806.
40. Rasmussen LS, Christiansen M, Johnsen J, et al. Subtle brain damage cannot be detected by measuring neuron-specific enolase and S-100beta protein after carotid endarterectomy. J Cardiothorac Vasc Anesth. 2000; 14(2): 166–170, doi: 10.1016/s1053-0770(00)90012-0, indexed in Pubmed: 10794336.
41. Mussack T, Hauser C, Klauss V, et al. Serum S-100B protein levels during and after successful carotid artery stenting or carotid endarterectomy. J Endovasc Ther. 2006; 13(1): 39–46, doi: 10.1583/05-1656.1, indexed in Pubmed: 16445322.
42. Terlecki P, Pawlik P, Iwaniuk A, et al. Carotid surgery affects plasma kynurenic acid concentration: a pilot study. Med Sci Monit. 2014; 20: 303–310, doi: 10.12659/MSM.890212, indexed in Pubmed: 24561546.
43. Ilzecki M, Ilzecka J, Przywara S, et al. Serum carnosine dipeptidase 1 and ubiquitin C - terminal hydrolase L1 as markers of brain damage in patients after carotid endarterectomy. Ulutas Med J. 2016; 2(3): 132, doi: 10.5455/umj.20160713014033.
44. Ilzecki M, Przywara S, Ilzecka J, et al. Serum microtubule associated protein tau and myelin basic protein as the potential markers of brain ischaemia-reperfusion injury in patients undergoing carotid endarterectomy. Acta Angiologica. 2016; 22(2): 37–43, doi: 10.5603/aa.2016.0008.
45. Iłżecki M, Iłżecka J, Przywara S, et al. Effect of carotid endarterectomy on brain damage markers. Acta Neurol Scand. 2017; 135(3): 352–359, doi: 10.1111/ane.12607, indexed in Pubmed: 27126899.
46. Ilzecki M, Przywara S, Ilzecka J, et al. Serum glial fibrillary acidic protein as a marker of brain damage in patients after carotid endarterectomy. Acta Angiologica. 2016; 22(1): 1–4, doi: 10.5603/aa.2016.0001.
47. Iłżecki M, Iłżecka J, Przywara S, et al. Serum neuron-specific enolase as a marker of brain ischemia-reperfusion injury in patients undergoing carotid endarterectomy. Acta Clin Croat. 2016; 55(4): 579–584, doi: 10.20471/acc.2016.55.04.07, indexed in Pubmed: 29117648.