Rīgas Stradiņa Universitāte

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S100 PROTEIN AND NEURON SPECIFIC ENOLASE AS DIAGNOSTIC AND PROGNOSTIC BIOMARKERS OF CEREBRAL INFARCTION

Summary of promotion thesis
to get a medical doctor's degree

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ABBREVIATIONS

AsyICH-asymptomatic intracerebral hemorrhage

CI - cerebral infarction

CT-computer tomography examination

HT- hemorrhagic transformation

IVT- intravenous thrombolysis therapy with recombinant human tissue plasminogen activator (rt-PA)

ICH- intracerebral hemorrhage

IQR- interquantile range

K-S tests- *Kolmogorov-Smirnov test*)

mRS- modified Rankine scale

NSE- neuron specific enolase

NIHSS- National Health Institute strole scale

SyICH- symptomatic intracerebral hemorrhage

SD- standad deviation

TOAST- *Trial of Org 10172 in Acute Stroke Treatment*

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INTRODUCTION

Stroke is one of the most topical problems of contemporary medicine. It is the most common cerebrovascular pathology and one of the chief mortality, demence and disability causes in the world (17; 25). In reference to WHO data, every year 15 million people suffer from cerebral infarction and 5 million people experience a deep functional defect (29;37). According to the National Health Service data in 2011, the mortality rate from stroke constitutes 116/100 000 people. (44)

Cerebral infarction or ischemic stroke is the problem which is encountered by a neurologist every day. In such a case it is essential to diagnose the cause of cerebrovascular disease as early as possible, assessing its severity and progress when choosing the most optimal treatment possibilities for the patient. Despite the fact that the diagnostics of cerebral infarction has been remarkably developed and has been very useful in the patient treatment, there are situations, however, when in the clinical practice we cannot sufficiently precisely determine the thereapeutic tactics and to choose the most appropriate treatment method.

In the last years the cerebral tissue lesion markers have been paid great attention to, because biomarkers can ease the assessment of cerebroischemic damage in cerebral infarction patients in the acute stage, thus identifying patients with a possible unfavourable disease outcome. (3; 4; 18) S 100 protein and NSE are cerebral lesion markers, which are most commonly used in clinical and experimental research. (10; 23; 36) S 100 protein is glia's specific Calcium ion-binding single dimer protein which regulates the cell growth processes (neurotrophic factor), participates in cell cycle regulation, cell contraction, movement, transcription and cell differentiation processes (22; 24).

NSE is glycolytic enzyme ecolase dimeric isoenzyme, which is mainly found in neurons and neuroendocrine cells (16; 30). Both biomarkers are considered to be the main specific neurobiochemical cerebral lesion markers in cerebral infarction and cerebral trauma (41; 42). In the studies of late it has been found that in patients with an acute cerebral ischaemia, in the first hours after the event, one can diagnose an increased biochemical substance level in blood and cerebrospinal fluid, which is due to the size of cerebral substance damage, clinical condition and the disease outcome (5; 9; 12; 18). The opinions exist that determination of markers can give a valuable extra information, more precisely identifying the diagnosis of the disease and the tactics of treatment in early disease stages (3; 31). In the literature the most commonly studied is S 100 protein and NSE, being the most useful and comparatively cheap, available, easily detectable and the easily interpretable (4; 11; 13; 23) results of analysis. Considering the above-mentioned, the promotion thesis has summarized several parameters simultaneously in which the prognostic usefulness of biomarkers is specified.

AIM OF STUDY

To evaluate S 100 protein and NSE as diagnostic and prognostic biomarkers of cerebral infarction, drawing conclusions as to their usefulness in daily practice.

OBJECTIVES OF STUDY

1. To evaluate S 100 protein and NSE level in patients with acute cerebral infarction and the correlation with the patient's functional status.
2. To determine S 100 and NSE correlation with radiological finding – infarction volume and hemorrhagic transformation rate.
3. To determine S100 protein and NSE correlation with thrombolytic therapy and thrombectomy effectivity, hemorrhagic complication rate.
4. To evaluate the possibility to use S100 protein and NSE cerebral infarction in diagnostics and prognosis of the disease outcome.

NOVELTY OF STUDY

Biomarkers were selected on the basis of general analysis of the acquired results, which allow to prognose the severity of the disease and the possible risk of complication development as a result of the specific therapy applied. For the first time a study was carried out in Latvia, in which S 100 and NSE prognostic value and the efficacy of thrombectomy procedure in relation to cerebrovascular pathology were specified. The aim and objectives set for the study summarize single research tasks in the world, which more evidently help assess the biomarkers from different aspects.

THEORETICAL AND PRACTICAL SIGNIFICANCE OF STUDY

By proving the prognostic value of biomarkers in diagnostics of cerebral infarction, in the choice of the disease outcome and applied

treatment method, the possibilities are improving to select the patients for specific therapy, prognosing its effectivity and decreasing the possible risk of complication development..

Specifying the importance of biomarkers in cerebral infarction, it would give a chance to investigate their pathophysiological release mechanism more purposefully, improving the understanding of cerebral infarction pathogenesis.

STRUCTURE AND VOLUME OF STUDY

The promotion thesis is written in the Latvian language. It consists of annotation, introduction, literature survey, materials and methods, work results, discussion, conclusions, practical recommendations and the literature sources used. The total volume of scientific work is 109 pages, analytically illustrated material depicted in 11 table, 32 pictures. The list of literature contains 148 references.

1. STUDY MATERIAL AND METHODS

The promotion work has been done at Riga Stradiņš University clinical grounds of Neurology and Neurosurgery Department – Pauls Stradins State University Hospital Neurology clinic in cooperation with the hospital's Clinical Immunological Centre and Radiology Institute. For doing the promotion study the confirmation was received from Rīga Stradiņš University Ethics Committee (decision Nr.E-9(2), 11.02.2010.).

1.1.**Study methodology and principles of group selection**

In the study data base were included 504 patients' medical information in the questionnaire worked out for the study:

- 336 patients with acute cerebral infarction
- 168 control group patients with non-inflammatory spinal pathology who were treated in Neurology Clinic

Inclusion and exclusion criteria of patient groups (N=336)

Inclusion criteria:

- acute cerebral infarction
- till 24 hours from the onset of cerebral infarction symptoms

Exclusion criteria:

- neurodegenerative CNS diseases
- oncological diseases in case history

Inclusion and exclusion criteria of control group (N=168)

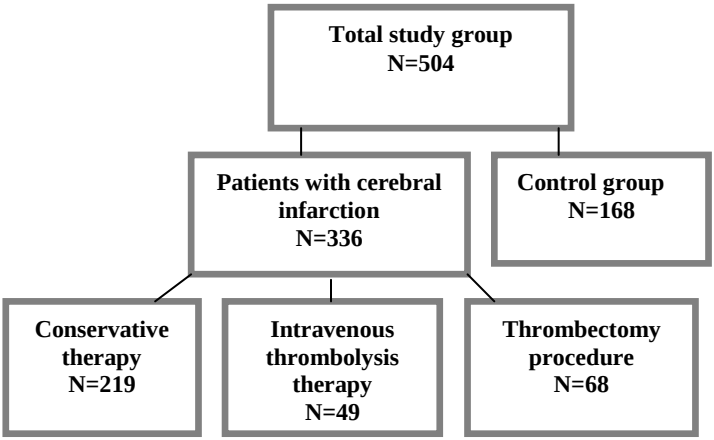
Inclusion criteria:

- there is no cerebral infarction
- there are data on cerebral infarction in the case history

Exclusion criteria:

- neurodegenerative CNS diseases
- oncological diseases in the case history

Distribution of patients into groups is shown in Picture 1.1.



Picture 1.1. **Distribution of study groups**

1.2**. Assessment of patients' functional status**

Patients' functional status was assessed by a certified doctor-neurologist, using a modified Rankine scale (mRS) and the National Health Institute stroke scale (NIHSS) on admission and discharge from the hospital.

1.3. **Radiological diagnostics**

Patients under study with CI on admission to hospital underwent CT (computer tomography) examination of the brain.A repeated visual imaging diagnostic radiological examination for the brain was done to patients during the treatment time:

- 24 hours after intravenous thrombolysis therapy with rtPA (recombinant human tissue plasminogen activator)
- 24 hours after thrombectomy procedure
- Neurological condition getting worse in dynamic
- On admission to hospital in CT (native) examination CI was not visualized

On the basis of radiological findings in the brain, the analysis was done of the cerebral infarction volume and the patients were divided into subgroups: CT negative CI subgroup, lacunary CI subgroup, partial CI subgroup, total CI subgroup.

1.4. **Analyses of biomarkers**

The concentration of biomarkers under study (S100 protein and NSE) was determined at Pauls Stradins State University Hospital Clinical Immunology Centre.

The analysis of patients with CI was done up to 24 hours since the beginning of the onset of CI symptoms.

Biomarkers were determined in the examined patient's serum, using the electrochemical luminiscense immunological test „ECLIA", and *Elecsys 2010* imunological test analyzer (*Roche Diagnostics GmbH*). In the test were used monoclonal antibodies against specific functional protein.

1.5. **Data statistical analysis**

The data acquired within the study period were recorded in a specially designed questionnaire and electronic data base.

The acquired qualitative and quantitative variables were processed, using the descriptive and analytical statistical methods.

Descriptive statistics.. Describing the distribution of variables in the ratio scale (central tendency, dispersion), there was used the mean arithmetic value and Standard deviation (for normally distributed data) or median value and interquartile range (IQR) (for asymmetric data).

Analytical statistics. Conformity of variables of the ratio scale to the normal probability distribution was tested by Kolmogorov-Smirnov (K-S) test. For confirmation or rejection of statistical hypothesis on equation of central tendency characteristics in normally distributed ratio scale variables the Student's test was used, for asymmetrically distributed – non-parametric methods, depending on the number of the groups to be compared: Mann-Whitney test, Kruskal Wallis test. The correlation of values was assessed using Spearman's rank correlation coefficient.For the calculation of statistical analysis there was used a specialized statistical programme (*SPSS 15 for Windows, SPSS, Chicago, IL*). Bilateral p value <0,05 was accepted as the threshold of statistical significance.

2. **STUDY RESULTS**

In accordance with the objectives of the work, systematized and statistically processed patients' demographic, epidemiological, clinical and laboratory data were acquired as a result of the study.

2.1. **Demographical and and clinical characteristics of study population**

For patients with CI under study (n=336) demographical characteristic parameters were analyzed which are showh in Table 2.1.

In the control group (n=168) there were 81 (48%) women and 87 (52%) men. The mean age of control group patients was 62,3 ± 6,3 years.

By distribution by gender and age the control and study group can be considered as equals (p< 0,05).

Table 2.1.

Demographical characteristics of patients with cerebral infarction

Characteristic parameter	***Measurement unit, presentation type***	***Value***
Gender	Women, n (%)	169 (50,3%)
	Men, n (%)	167 (49,7%)
Age	years, mean (±SD)	65,5 (±9,3)

Assessing the dynamics of the neurological status, the patients' functional status on admission and discharge was estimated by mRS and NIHSS scales, which are shown in Table 2.2. Values of both scales are compared on admission and discharge. Study results showed that the improvement of patients' functional status during the treatment – assessing by mRS per 1 point, but by NIHSS per 6 points, as well as on discharge – still showed the existing disability of patients. The majority (92%) of patients with CI included into the study did not have serious functional disorder till the hospitalization, which was shown by mRS mediana, the value of which was 0.

Table 2.2.

Characteristics of functional status in patients with cerebral infarction

Functional scales	***Measurement unit, presentation type***	***Value***	***P value***	***Statistical method***
NIHSS on admission	NIHSS ppoints median value (IQR)	14(11-16)	p<0,001	K-S test
NIHSS on discharge	NIHSS points median value (IQR)	8 (5-13)	p<0,001	K-S test
mRS prior to hospitalization	mRS points median value (IQR)	0	p<0,001	K-S test
mRS on admission	mRS points median value (IQR)	2 (2-4)	p<0,001	K-S test
mRS on discharge	mRS points median value (IQR)	1 (1-2)	p<0,001	K-S test

Length of hospital treatment of patients with CI is shown in Table 2.3.

Table 2.3.

Leangth of hospital treatment of patients with cerebral infarction

Hospital treatment	***Measurement unit, presenation type***	***Value***	***P value***	***Statistical method***
Neurological clinic	day, median value (IQR)	10 (8-13)	p<0,001	K-S test
Stroke unit	day, median value (IQR)	5 (3-5)	p<0,001	K-S test

In the study the patients were analyzed CI using TOAST criteria, depicted in Table 2.4. Part of patients had both atherothrombotic and cardioembolic CI risk factors and they, in correspondence to TOAST criteria, were included into a separate – unspecified etiology CI subgroup.

Table 2.4.

Distribution of study group with cerebral infarction in correspondence to TOAST criteria

TOAST classification	Number of patients	
	n	%
Cerebral infarction of cardioembolic genesis	113	33,6
Cerebral infarction of atherothrombotic genesis	102	30,4
Cerebral infarction due to small blood vessel pathology	23	6,8
Other cause	8	2,4
Unspecified etiology	90	26,8
In total	336	100

Assessing the CI volume in patients, visual radiological imaging diagnostic method on admission and during the treatment was introduced– patients were divided into subgroups. On admission one third of patients who underwent the radiological diagnostics did not have findings, which point to the fact, that one could judge about the potential brain lesion only by the assessment of the patient's functional status. Patient distribution according to the visual diagnostic results is shown in Table 2.5.

Table 2.5.

Patient distribution by visual diagnostic results

Radiological cerebral infarction subgroups	***Visual imaging diagnostics on admission to hospital***		***Repeated visual imaging diagnostics in dynamics***	
	n	**%**	**n**	**%**
CT negative finding	93	27,7	9	2,7
Lacunar CI	57	17,0	61	18,2
Partial CI	137	40,8	201	59,8
Total CI	49	14,6	65	19,3
In total	336	100	336	100

During the hospital treatment 19 study patients (6%) with CI were observed to have the worsening of the neurological status. This patient group underwent a repeated CT examination of the brain, where hamorrhagic transformation (HT) was identified. HT incidence in the study patients with CI in the radiological subgroups is shown in Table 2.6.

Table 2.6.

Incidence of hemorrhagic transformation in radiological cerebral infarction subgroups (summarized results of visual imaging diagnostics)

Cerebral infarction radiological subgroups	***Hemorrhagic transformation***			
	with		**without**	
	n	**%**	**n**	**%**
CT negative finding	9	2,8	0	0
Lacunar CI	61	19,2	0	0
Partial CI	189	59,6	12	63,2
Total CI	58	18,3	7	36,8
In total	317	100	19	100

2.2. **Level of biomarkers in patients with cerebral infarction and control group**

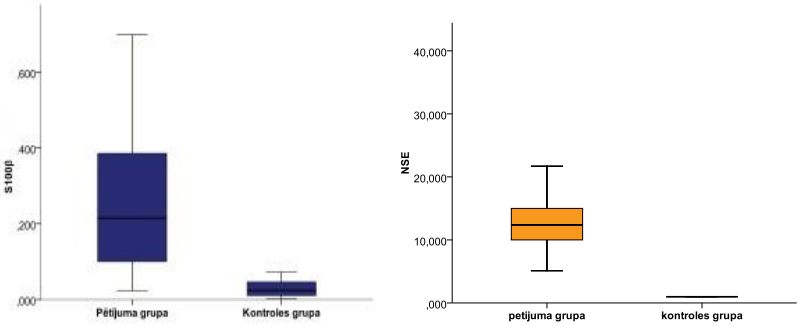
In the studied patients with acute cerebral infarction the level of S 100 protein and NSE in serum statistically significantly differed in comparison to the control group. The values of the observed studied biomarkers are shown in Table 2.7. and Picture 2.1.

Table 2.7.

Level of biomarkers in the studied population

	Patients with cerebral infarction =336)	***Control group (n=168)***	***P value***	***Statical method***
S100 protein, median (IQR)*, µg/L	0,215 [0,100-0,386]	0,024 [0,011-0,046]	p<0,001	*Mann-Whitney U* test
NSE, median (IQR)*, µg/L	11,95 [9,63-15,08]	1 [1-1]	p<0,001	*Mann-Whitney U* test

*IQR-interquartile range (25./75. percentiles)



Picture 2.1. **S100 protein and NSE level (µg/L) in patients with CI and control group**

2.3. Correlation of biomarkers with functional status of patients with cerebral infarction

Patients with CI were analyzed the biomarker level in association with patients' functional status (NIHSS scale) on admission and discharge from the hospital.

The study results showed that a higher S100 protein and NSE concentration in serum in patients with cerebral infarction is connected with a worse clinical functional prognosis. Assessing both biomarkers in association with the functional status, similar correlation coefficients were found on admission and discharge from the hospital, which is shown in Table 2.8.

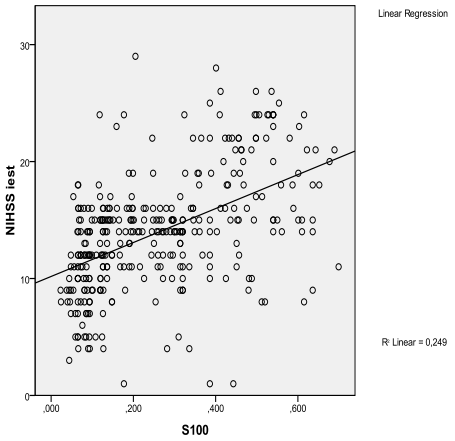
Comparing S100 protein with NSE level in correlation with a patient's functional status, we could conclude, that in the serum they statistically significantly positively correlated with the severity of the patient's neurological status on admission and on discharge, however, S100 protein correlated with the functional status better than NSE on admission, which is shown in Picture 2.2. and 2.3.

Table 2.8.

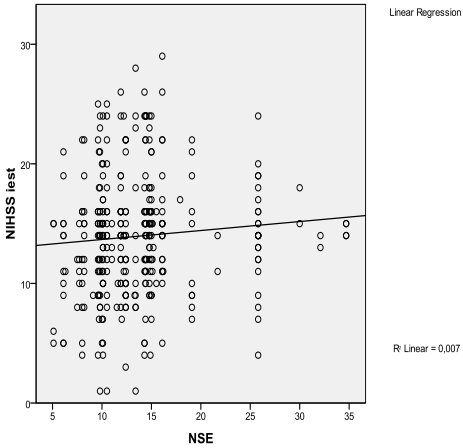
S100 protein and NSE level correlations with the functional status

Functional status	r_{s} *	
	S100 protein	**NSE**
NIHSS on admission	0,514	0,112
	(p<0,001)	(p=0,04)
NIHSS on discharge	0,501	0,122
	(p<0,001)	(p=0,026)

* r_s- *Spearman's rank correlation coefficient*



Picture 2.2. **S100 protein level correlation with NIHSS scale on admission to hospital**



Picture 2.3. **NSE level correlation with NIHSS scale on admission to hospital**

2.4 **Correlation of biomarkers with the volume of cerebral infarction**

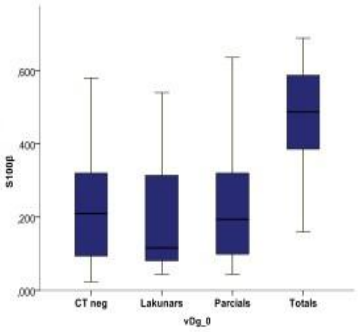
Patients with CI were assessed S100 protein level in serum in relation to CI volume and a higher S100 protein concentration was in patients with total CI, which is shown in Table 2.9. and Picture 2.4. (*Kruskal Wallis* test). We have to mention that a comparatively high S100 protein level was in patients with CI, who on admission after CT performance were not visualized cerebral infarction (CT negative finding).

Table 2.9

S100 protein level in radiological cerebral infarction subgroups

Radiological CI subgroups	***S100 protein median,(IQR) * (μg/L) p<0,05***
CT negative finding	**0,210 [0,094-0,320]**
Lacunar CI	**0,116 [0,081-0,314]**
Partial CI	**0,194 [0,099-0,320]**
Total CI	**0,487 [0,386-0,587]**

*IQR- interquartile range (25./75.percentiles), *Kruskal Wallis* test



Picture 2.4**. S100 protein level (μg/L) on admission in hospital in cerebral infarction subgroups**

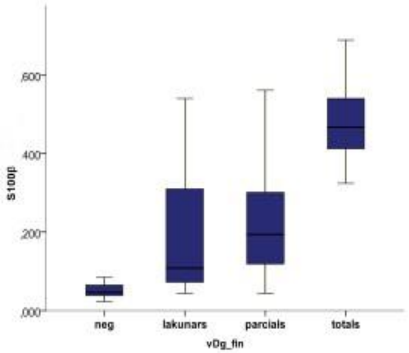
In the study, in order to improve the patients' group selection, a repeated CT examination in dynamics was done for patients with negative CT finding on admission. After a repeated CT examination in a majority of patients a kind of CI radiological subgroups had been formed, thus supplementing radiologically divided CI subgroups, changing S100 protein median value distribution, which is shown in Table 2.10.and Picture 2.5., in which were compared all 4 radiological subgroups (*Kruskal Wallis* test). Assessing S100 protein level after the repeated examination for CT negative group, the study result demonstrated an essentially higher S100 protein level in patients with total CI.

Table 2.10.

Distribution of radiological cerebral infarction subgroups after repeated examination

Radiological CI subgroups	***S100 protein median,(IQR) * (μg/L) p<0,05***
CT negative finding	0,046[0,038-0,065]
Lacunar CI	0,108[0,072-0,310]
Partial CI	0,194[0,119-0,301]
Total CI	0,467[0,412-0,540]

*IQR- intraquartile range (25./75.percentiles), *Kruskal Wallis* test



Picture 2.5**. S100 protein level (µg/L) in cerebral infarction subgroups after repeated examination**

In the study NSE level median for patients with cerebral infarction on admission to hospital between CI radiologic subgroups did not essentially differ, which is shown in Table 2.11. and Picture 2.6.(*Kruskal Wallis* test).

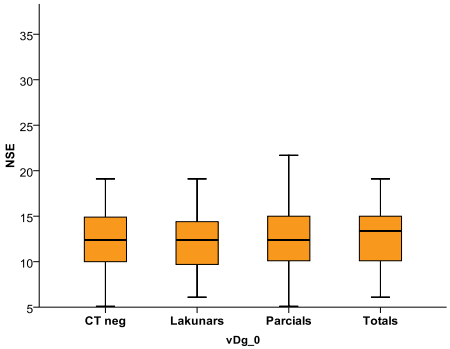
After the repeated CT examination of the patients with CT negative finding no essential changes were observed in NSE median value distribution.

Table 2.11.

NSE level in radiological cerebral infarction subgroups

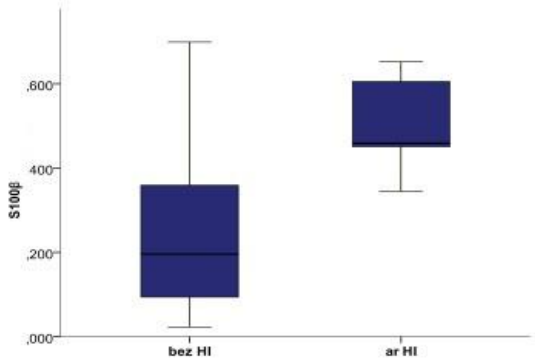
Radiological CI subgroups	***NSE median,(IQR) * (µg/L) p<0,05***
CT negative finding	12[10-15]
Lacunar CI	12[10-14]
Partial CI	12[10-15]
Total CI	13[10-15]

*IQR- interquartile range (25./75.percentiles), *Kruskal Wallis* test



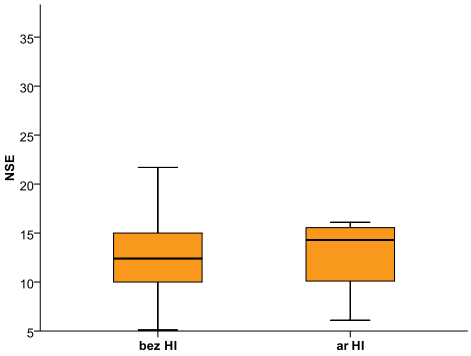
Picture 2.6. **NSE level (µg/L) on admission to hospital in various cerebral infarction radiological subgroups**

Comparing S100 protein level in the studied patient group in which hemorrhagic transformation had developed with the patient group in which it had not developed, there were seen statistically significant differences, $p<0,05$, *(Mann-Whitney U* test), shown in Picture 2.7. It shows that the patients with CI, who on admission had a higher S100 protein level, may have a higher HT development risk in dynamics.



Picture 2.7. **S100 protein level (µg/L) in relation to hemorrhagic transformation**

Comparing NSE level in patients who developed HT in dynamics with the patient group where it did not develop in dynamics, there were not observed statistically significant differences of NSE level, which are shown in Picture 2.8., p>0,05, (*Mann-Whitney U* test).



Picture 2.8. **NSE level (µg/L) in relation to hemorrhagic transformation**

2.5. **Correlation of biomarker level with thrombolysis efficacy and development of hemorrhagic complications**

In the study from the total patient group with cerebral infarction, 49 patients (14,6%) were done intravenous thrombolysis with rtPA (IVT).

Thrombolysis therapy was effective for 55,1 % patients, for 44,9% thrombolytic therapy was ineffective. IVT was considered to be effective if a patient's functional status improved by 2 points according to functional assessement scale.

The study results showed that by stating S100 protein level in patients with CI it is possible to prognose IVT efficacy. S100 protein level differences between the effective and ineffective IVT group is shown in Table 2.7. and Picture 2.9.

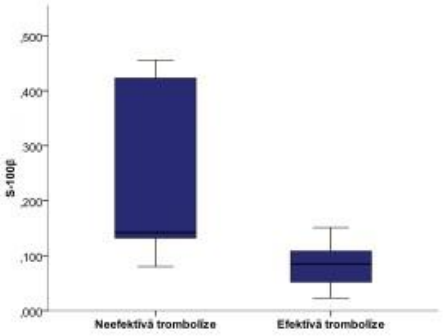
Comparing the NSE level between the patient groups by effective and ineffective IVT, there as not observed astatistically significant difference between them,as seen in Table 2.7.

Table 2.12.

Level of biomarkers efficacy of intraveous thrombolytic therapy

IVT efficacy	***S100 protein median* (µg/L) p<0,05***	***NSE median*(µg/L) p>0,05***
Ineffective	0,142 [0, 132-0,423]	13 [11-15]
Effectivity	0,085 [0,048-0,122]	12 [10-14]

*****IQR- interquartile range (25./75.percentiles), *Mann-Whitney U* test



Picture 2.9. **S100 protein level (μg/L) in relation to intravenous thrombolysis efficacy**

Analyzing in the study the development of hemorrhagic complications after IVT therapy, which is shown in Table 2.8. the study results demonstrate, that from 49 patients, who were done IVT, 37 patients after IVT therapy were not observed hemorrhagic complications, while 9 patients from the study group developed intracerebral hemorrhagic complications, 3 patients were observed extracranial bleeding.

Table 2.13.

Development of complications after intravenous thrombolysis therapy

Complications after intravenous thrombolytic therapy	***Hemorrhagic complication rate***	
	N	**%**
Without complications	37	80,5
Symptomatic intracerebral hemorrhage	3	6,5

2.13. Table continued

Complications after intravenous thrombolytic therapy	***Hemorrhagic complication rate***	
Asymptomatic intracerebral hemorrhage	6	13

*3 patients had bleeding from other organs

The study results showed that the higher S100 protein concentration observed was in the group with symptomatic intracerebral hemorrhage. It points to the fact that patients with a higher S100 protein level on admission to hospital, may have a greater intracerebral hemorrhage development risk after IVT therapy (see Picture 2.10.). NSE level between groups did not essentially differ. Level of biomarkers between the patient groups with ICH and without it is shown in Table 2.9.

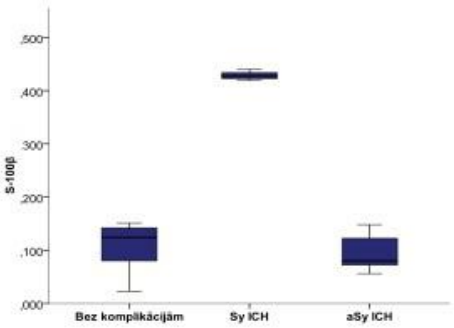
Table 2.14.

Level of biomarkers in relationship to complications of intravenous thrombolysis therapy

IVT complications	***S100 protein (µg/L) p<0,05****	***NSE (µg/L) p>0,05****
No complications (N=37)	0,124 [0,080-0,142]	12 [10-15]
Symptomatic ICH** (N=3)	0,42 [0,419-0,440]	14 [10-15]
Asymptomatic ICH** (N=6)	0,080 [0,072-0,122]	10 [10-12]

*****IQR- interquartile range (25./75.percentiles), *Kolmogorov-Smirnov* test

**ICH- intracerebral hemorrhage



Picture 2.10 **S100 protein level (μg/L) in relationship to intracerebral hemorrhagic complications**

2.6. **Correlation of biomarker levels with the result after thrombectomy**

Up to now in the literature there are not described the biomarkers in relation to thrombectomy. In our study, for the first time, there are demonstrated the results, in which we observed a statistically significant S100 protein level difference in patients with a positive dynamics after thrombectomy.

In the study 68 patients with CI were done thrombectomy procedure.Patients' functional status before and after the procedure was assessed by NIHSS scale, the mean number of points, according to NIHSS scale before thrombectomy, was 16, while after the procedure – 10 points. Like a positive dynamics after thrombectomy was considered the improvement of the patient's functional status according to NIHSS scale by 2 points.

After thrombectomy procedure 45 patients (66%) from 68 patients groups were discharged with the improvement of functional status, while in 20 patients (34%) on discharge, the functional neurological status was without essential dynamics, in 3 patients on discharge the functional neurological status was without essential dynamics, 3 patients were observed to have the worsening of the status.

The study results showed that in patients with effective thrombectomy, S100 protein median value in comparison to ineffective thrombectomy patient group, statistically significantly differed ($p<0,05$, *Mann-Whitney U* test). Our study results allow us to conclude, that by determining S100 protein level in serum for patients in the clinical practice before the invasive thrombectomy manipulations, one can prognose its outcome. NSE concentration in serum before the manipulation, in its turn, did not demonstrate a statistically significant difference in patients with positive dynamics ($p>0,05$, *Mann-Whitney U* test). Clinical outcome in relationship with biomarker level in patients after thrombectomy procedures is shown in Table 2.10.

Table 2.15.

S-100 protein and NSE level (μg/L) in thrombectomy patient groups

Clinical outcome after thrombectomy	***S100 protein median**** *(μg/L), p<0,05*	***NSEmedian**** *(μg/L), p>0,05*
Negative dynamics/without dynamics	0,346 [0,281-0,402]	12 [10-13]
Positive dynamics	0,148 [0,081-0,192]	11 [10-14]

*IQR-interquartile grade (25./75.percentiles), *Mann-Whitney U* test

DISCUSSION

The current study analyzes S100 and NSE diagnostic significance in patients with cerebral infarction in relation to the brain lesion size, the patient's functional status, radiological finding and the efficacy of modern cerebral infarction therapy, specifying its prognostic effectiveness in case of cerebral infarction.

Comparing the characteristic demographical parameters acquired in our study to the ones reported in the literature, no significant differences were noticed, which witnesses on the similarity of the population under study, laboratory diagnostics and therapeutic methods, and gives a chance to compare the data acquired in the study with those in other authors' publications (5; 13; 23; 38; 41).

S100 protein and NSE level may increase at different comorbidities, therefore in the selection of total study population we did not include patients with neurodegenerative and oncological diseases in the case history. Other authors' described studies demonstrate similar inclusion and exclusion criteria like those used in our study (1; 23; 41;42).

In the study the patient population in relation to the age and gender was homogenous. In the patient group with CI and control group no significant demographical parameter differences were noticed, which excludes the influence of the age and gender on the parameters under analysis. At present a clearly defined biomarker concentration in relation to an individual's age and gender has not been proved (38).

In the study the distribution of patients by their CI etiopathogenetic type was homogenous, not emphasizing one specific subgroup.The study group patients were mainly divided into 3 bigger subgroups by TOAST.

33,6% patients with cardiothrombolic genesis CI type, 30,4 % patients with atherothrombotic genesis CI type and 26,8% unspecified genesis CI type, in which were included patients with atherothrombotic and cardioembolic CI risk factors.

Analyzing the treated patients' functional status before and after CI, one can conclude, that the patients' functional status during the treatment had improved (according to NIHSS by 5 points, according to mRS by 1 point), however, at the moment of discharge from hospital a part of patients had a lasting functional incapacity. These study results prove that CI is a serious disabling disease which disintegrates the patients from the society and makes us look for new ways, by improving the present CI diagnostic methods, how to apply more advanced methods in treatment of CI.

Patients with CI were observed to have statistically significant differences in the concentration of studied biomarkers, in comparison to the control group patients, which is similar to the current authors' publications (13; 19; 23; 26; 41).

Analyzing the markers level association with NIHSS scale, the study results demonstrated both markers' positive correlation with the functional status on admission and on discharge, and by comparing them both S100 protein correlated better than NSE, however, one should say, that by analyzing these correlations, they were seen to be comparatively weak, which is shown by the correlation coefficients seen in the results. Explaining the fact from the point of view of the topographic theory of cerebral tissue anatomy, markers should correlate to the size of damaged tissues rather than the functional status, i.e., a slight brain lesion can cause markedly severe clinical syndroms with a high risk of the existing functional status (for example, infratentorially localized CI – in the brain stem). The brain is

structurally more complicated than the heart, because it has multiple neuron and glia cell types. The brain structure is not homogenous, the brain cells possess various degree heterogenecity and functionality, they have different sensitivity on the ischemic lesion, depending on its localization, as well as tolerance of various lesion mechanisms, therefore one should remember, that the quantitative indices of biomarkers not always characterize the patient's functional incapacity. (3; 9)

So far the current authors' studies demonstrate that by identifying S100 protein and NSE level in patients at various time periods (after 24 hrs), one can observe a better biomarker correlation with the functional status and it being best seen just at the time of discharge from hospital (5;23; 41; 42).

In the literature the authors use different functional status assessment scales, but the most commonly used, however, are NIHSS and mRS, similarly to those used in our study (5; 41; 42).

For more precise data analysis, assessing the association of the functional status to CI size, we have used NIHSS scale, because it is a more precise functional status assessment scale in comparison to mRS.

Analyzing the association of the patient's functional status by NIHSS with brain lesion size, the study data showed, that in a part of patients the functional status with a wider ischemic lesion size (i.e. patients with total CI, partial CI) CT was remarkably more severe. One should mention, however, that in 27,7% patients, admitted to hospital with acute CI, it was not verified by the computer thomography examination. In this patient group, in an early CI phase, CI potential size could be identified by evaluating the patient's functional status clinically. In an early CI phase, in order to assess the brain tissue ischemic size, the CT native examination method not always is sufficiently informative, however, it is sensitive enough to verify the

intracerebral hemorrhage. The authors in the literature decribe CT diagnostic sensitivity in ischemic CI case being lesser than 33% (27; 43).

The before-mentioned gives evidence to the significance of biomarkers for confimation of acute CI diagnosis, thus, in hospitals, using biomarkers as a screening diagnostic method for confirmation of CI diagnosis there would be a possibility to decide on the necessity to apply multimodal neuroradiological diagnostics, in such a way optimizing the use of financially expensive examinations and to start a more appropriate acute CI therapy by earlier prognosing its outcome. (15)

In our study, comparing S100 protein level in radiological CI subgroups, a statistically significant difference was observed between them. The highest S100 protein concentration was in patients with total CI. One should mention, that a comparatively high was S100 protein level in patients with CT negative finding, therefore, in order to improve the selection of study patients, there was repeatedly done radiological examination in dynamics, during which a kind of group was approved.

In the literature are describe various study results in which the correlation of S100 protein level with CI size is shown, determining the marker level in later periods of time (after 24 hrs) (5; 7; 23; 42). The aim of our study was to analyze the prognostic value of markers in acute CI case up to 24 hrs, identifying them in serum in a single case.

Evaluating the correlation of NSE level with the brain lesion size in patients with acute CI in our study, no statistically significant results were acquired. In the literature there is also described the association of NSE level with CI size, emphasizing that by stating NSE level in a later period of time after the onset of CI, there exists its better correlation with CI size (2).

In our study is statistically significantly shown the correlation of S100 protein level in patients with CI, who in dynamics were developing hemorrhagic transformation (6%), which is the grounds for the prognostic usefulness of S100 protein in the development of hemorrhagic transformation (HT).

On the other hand, in our study the NSE level did not significantly differ in the patient group with HT and without it. It points to the fact, that NSE is not a prognostic indicator in HT development in patients with CI.

In the study the intravenous thrombolytic therapy with rtPA (IVT) was used in 14,6 % patients (n=49), which is a higher indicator than the one seen in the clinic in 2010 - (11,5%), which could be explained by a selection of specific representative group for the study.

In the study there was specified S100 protein prognostic usefulness, determining it before IVT. In the effective thrombolysis patient group, S100 protein level was statistically significantly lower than in ineffective thromblysis patient group, i.e., in the group in which there developed hemorrhagic complications. The acquired data demonstrate the correlation of S100 protein level with HT development risk in patients after the existance of thrombolysis and hematoencephalic barrier damage and its significance in early stages of cerebral ischemia, which allows to conclude that S100 protein is HEB disfunction marker.

The literature also decribes HT development after IVT in relation to HEB dysfunction, emphasing that S100 protein is HEB dysfunction marker (6; 8; 14; 20; 21; 28).

In our study we analyzed more in detail also S100 protein level association with posthrombolytic hemorrhagic complication type, demonstrating S100 protein's higher concentration in the group with

sympatomatic intracerebral hemorrhage. We still have to say that the number of patients who developed intracerebral complications is not sufficient in order to draw conclusions on the connection of marker concentration with the type of the complication.

Foerch and coauthors also emphasizes that there is no essential difference in S100 protein concentration between hemorrhagic complications, mentioning that S100 protein is a qualitative HEB damage marker (8).

In the study NSE level in effective and ineffective thrombolysis group did not essentially differ. It could be explained by NSE different pathophysiological release mechanism and time in which NSE gets involved into CI during the ischemic process cascade. In the literature no cases are analyzed as to NSE level relation to IVT and the development of complications after it, therefore we do not have the data to compare to our study results.

Lately in our clinic there is being used invasive CI treatment of cerebral infarction – thrombectomy, which is an effective mechanical revascularization therapy method in restoration of blood flow in proximally occluded brain artery segments, providing the blood supply to potentially vital brain tissues. (33)

So far thrombectomy has not been included into the therapeutic guidelines for cerebral infarction., due to highly existing complication risk which can develop after the manipulation. One of the problems why the availability of this manipulation is limited in the clinical practice deals with unspecified patient selection.Correctly selected patient for this manipulation would reduce the possible risk for the development of postprocedural complications. (33; 34; 35).

In our study by determining S100 protein and NSE level in serum in 68 patients before the manipulation, the data acquired demonstrate, that in patients with CI before thrombectomy manipulation it is possible to prognose its outcome, prognosing the possible development of the complication risk. Lower S100 protein concentration is connected with a better functional outcome after thrombectomy. Assessing NSE level prognostic usefulness in patients, there was not found a statistically significant difference between the positive and negative outcome. So far in the literature no study data had been demonstrated in which S100 protein and NSE were investigated in connection with the outome of thrombectomy, therefore at present it is impossible to compare the acquired data results.

In the promotion study NSE prognostic effectiveness in relation to cerebral infarction was not observed.. This lack of association in the study could be explained by specificities of the research methodology – the time for obtaining the studt samples, which, perhaps, affected the NSE median value.

The hypothesis set for the study that S100 protein and NSE level correlate with the functional status in patients with acute cerebral infarction was confirmed. For both markers we managed to demonstrate the correlation with the severity of a patient's functional status. The rest of work hypotheses were confirmed too, in which S100 protein level correlates with radiological finding, the efficacy of thrombolytic therapy and thrombectomy and the incidence of hemorrhagic complications. Thus, the hypothesis of the study confirmed that S100 protein can be used in the clinical practice as a biomarker for diagnosis and prognosis of cerebral infarction, but prognostic effectiveness of NSE in the clinical practice has not been confirmed in the study.

CONCLUSIONS

S100 protein and NSE level is higher in patients with cerebral infarction in comparison to the control group patients, both markers correlate with the patient's functional status.

S100 protein level correlates with cerebral infarction size and can be considered the prognostic factor of the development of hemorrhagic transformation. NSE level is not associated with cerebral infarction size, as well as it is not prognostically useful in the assessment of hemorrhagic transformation risk.

Increased S100 protein level correlates with the efficacy of thrombolytic and thrombectomy therapy, as well as the incidence of hemorrhagic complications. NSE level is not associated with the efficacy of thrombolytic and thrombectomy therapy and the incidence of hemorrhagic complications.

Determination of S100 protein level can be recommended as a diagnostic and prognostic indicator. Determination of NSE level, is not enough information to apply in clinical practice.

PRACTICAL RECOMMENDATIONS

Taking into account the results acquired in the study, S100 protein can be recommended in the clinical practice as prognostically useful biomarker in acute cerebral infarction case. S100 protein is one of the complementary diagnostic possibilities in cerebral infarction case, improving

the patient selection for specific treatment methods, prognosing their efficacy and reducing the development of possible complication risk.

LIST OF PUBLICATIONS AND REPORTS ON THE STUDY THEME

1. **Priede Z.**, Ķēniņa V, Miglāne E., Millers A., Pūcīte E., Radziņa M. S-100 proteīns kā cerebrāla infarkta plašuma un iznākuma prognostisks marķieris. RSU zinātnisko rakstu krājums „Zinātniskie raksti: 2010. gada medicīnas nozares pētnieciskā darba publikācijas" Internās medicīnas sadaļā. 77.–86. lpp.
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3. **Priede Z.**, Ķēniņa V., Pūcīte E., Gudreniece A., Millers A. Neironu specifiskā enolāze (NSE) kā cerebrāla infarkta plašuma un iznākuma prognostisks marķieris. RSU zinātnisko rakstu krājums „Zinātniskie raksti: 2011. gada medicīnas nozares pētnieciskā darba publikācijas" Internā medicīnas sadaļā.33.-36.lpp.
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3. **Priede Z.**, Kenina V., Sabelnikova S., Pucite E., Miglane E., Millers A. Association of protein S100ß and NSE with Efficient Trombolytic therapy and Higher Risk of Hemorrhagic Transformation After Thrombolytic Therapy in Acute Stroke. Abstract, poster presentation XXI. European Stroke Conference. 2012.

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1. Millers, Miglāne E., Ķēniņa V., **Priede Z.**, Lapinska I. S-100 proteīns un NSE kā cerebrāla infarkta prognostiskie marķieri, to korelācija ar insulta riska faktoriem, išēmiskā bojājuma lielumu un trombolītiskās terapijas efektivitāti. RSU Zinātniskā konference, 2009; 81. lpp., stenda referāts.
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