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Disturbance of thiol/disulfide aminothiols homeostasis in patients with acute ischemic stroke stroke: Preliminary findings

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ABSTRACT

Objectives: To determine the disruption of low-molecular-weight aminothiols (LMWTs: cysteine, cysteinylglycine, homocysteine, and glutathione) homeostasis in blood plasma during the acute and early subacute stages after ischemic stroke.

Patients and methods: We admitted 41 patients with primary large-artery atherosclerosis and cardioembolic stroke in the carotid arteries within the first 6–24 h from the moment of neurologic symptoms development. We included 31 patients with chronic cerebral ischemia in the control group. Total LMWT levels and their reduced forms were measured in blood plasma on the 1st, 3rd, 7th, and 15th days after stroke.

Results: Our study demonstrated a decrease of cysteine and cysteinylglycine reduced forms and an increase of total glutathione and cysteine levels. There were no differences in LMWT levels among stroke subtypes (large-artery atherosclerosis and cardioembolic stroke). The decrease (or increase) in GSH and Hcy redox status on the 3rd day after stroke was associated with severe neurological deficit. Total Hcy (1st day), Cys (3rd day) and CG(7th day) levels were associated with the size of cerebral infarction area. Logistic regression analysis indicated that reduced homocysteine, total cysteinylglycine levels, and cysteine redox status at admission were predictive factors for ischemic stroke occurrence with a probability of 86.2% ($p < 0.001$).

Conclusions: LMWTs may indicate the severity of neurological deficit and the size of the cerebral infarct, and their complex determination can be of diagnostic importance both at an early stage of ischemic stroke development and during its monitoring.

1. Introduction

Stroke continues to be a major medical and social problem because of its large part in the structure of population morbidity and mortality, which are significant indicators of temporary labor losses and primary disability.

The past decade has witnessed tremendous achievements in the ability to diagnose stroke, but its treatment remains unsatisfactory. Stroke remains the second most common cause of death worldwide, particularly in the elderly. The early mortality rate of stroke is as high as 10–12% and it remains higher for several years after the acute event in stroke patients than in the general population [1]. Stroke is also the fourth leading cause of disability-adjusted life-years around the world [2]. Since life expectancy is continuing to grow, the absolute number of individuals with stroke will increase further.

Oxidative stress (OS) plays an important role in the pathogenesis of nervous tissue damage in the presence of stroke [3]. The accumulation of reactive oxygen species (ROS) in the cerebral infarction area and penumbra leads to the damage of membranes, DNA, peptides and to the redox balance disruption of various biochemical systems including thiol containing compounds [4]. Apart from the local OS, brain damage also induces systemic OS through activation of sympathoadrenal system [5–7]. At the same time, massive release of ROS and proinflammatory cytokines from peripheral vessels occurs already during the acute

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period of cerebral ischemia. The particular aspects of the development of a generalized OS and its role in the development of stroke and its complications have not been studied enough. However, it has been suggested that this contributes to a hypertensive state and has a negative effect on the course and outcome of stroke [8].

This explains an interest in using of OS markers and indicators of various antioxidant system states in an organism for diagnostics and prognosis of stroke development. These markers include total oxidant status, total antioxidant capacity, levels of SH-groups (tSH), levels of 8-hydroxy-20-deoxyguanosine, thiobarbituric acid reactive substances, myeloperoxidase acivity, oxidized low-density lipoprotein and others [9,10]. Although in most cases of stroke significant changes of these indicators are observed their diagnostic and prognostic value remains unclear.

Several studies have shown that in acute stroke, tSH is significantly reduced [11–15], and its low level persists for several months after stroke [16]. Tsai et al. demonstrated significant differences in tSH between groups of patients with small- and large-vessel disease. It was also shown that this indicator has prognostic value [11]. However, the tSH level (about 1 μmol/L) was significantly lower compared with that in other studies. Musumeci et al. and Bektas et al. reported that tSH correlates with severity of stroke in the acute period [12,13]. However, Icme et al. did not find this correlation [14]. Even less information is known about the effect of acute stroke on the homeostasis of LMWTs. LMWTs (cysteine [Cys], glutathione [GSH], cysteinylglycine [CG], homocysteine [Hcy]) and others are important components of the blood antioxidant defense system. They are in a state of dynamic equilibrium between disulfide and reduced (*r*) forms. The ratio of the reduced forms to the total content of each thiol can be used to characterize the redox status (RS) [17]. Disulfide forms predominate in blood plasma; reduced fractions account for only 1–5% and constitute less than 10% of tSH in humans [18,19]. *r*LMWTs are the most chemically active fractions of tSH. High metabolic rate (turnover rate 1–2 min) and reactivity with ROS [20] make it possible for LMWTs to be used as markers of OS which are more sensitive than tSH.

Our recent studies using experimental models of cerebral ischemia have shown that in the first hours of ischemia, the RS and reduced forms of plasma LMWTs undergo a significant drop [21]. Furthermore, a more severe model of focal ischemia was accompanied by more pronounced impairment of LMWTs homeostasis than a less severe model of global ischemia. The use of beta-adrenergic antagonists prevented the disruption of LMWTs homeostasis in both blood plasma and ischemic brain tissue [22]. At the same time, to our knowledge, thiol/disulfide homeostasis of these metabolites in the blood plasma of patients in the early stages of cerebral ischemia has never been studied. Therefore, the aim of the present study was to reveal LMWT homeostasis disruption in blood plasma during the acute and early subacute stages after ischemic stroke.

2. Materials and methods

2.1. Patients

Our local Medical Research Ethics Committee approved this study. The main inclusion criteria were as follows: acute first large-artery atherosclerosis and cardioembolic stroke according to the TOAST (Trial of ORG 10172 in Acute Stroke Treatment) classification [23]; the first 24 h from the neurologic symptom onset; age 45–80 years; and informed consent signed by patients or their first-degree relatives.

The exclusion criteria were: a delay of more 24 h between onset of symptoms and diagnosis of ischemic stroke; lacunar infarct; undetermined causes of stroke; hemorrhagic stroke; epilepsy; pregnancy; type 1 diabetes; acute myocardial infarction; permanent cardiac pacing; decompensated renal, hepatic, or respiratory failure; congestive heart failure of a III–IV functional class; oncological diseases; and chronic inflammatory, autoimmune, and hematological disorders. Patients with any infectious disorder within 15 days after acute ischemic stroke were also excluded.

We included 41 patients with a mean age of 71.5 (range 59.0–78.0) years in the group of patients with primary large-artery atherosclerosis or cardioembolic stroke in the carotid arteries. Among these patients, 15 (37%) men and 26 (63%) women were admitted within the first 6–24 h from the moment of neurologic symptom development. Diagnosis was based on clinical data, neurological examination, and results of brain magnetic resonance imaging (MRI) with diffusion-weighted imaging (DWI), color duplex study of the brain arteries, and transthoracic echocardiography.

We included 31 patients with chronic cerebral ischemia in the control group matched for age and sex distribution (average age 69 [range 60–75] years; 12 [38.7%] men and 19 [61.2%] women). Chronic cerebral ischemia is currently defined as slow progressive deterioration of brain perfusion that leads to the diffused alteration of brain tissue and to impairment of its functions. The inclusion criteria were mild and moderate non crisis hypertension of over 5-year duration period in patients of both sexes from 45 to 80 years of age, lesions located in the subcortical structures, such as single small deep infarcts and/or moderate expansion of subarachnoid space according to the MRI combined with minimal changes in the neurological status. Neither the patients nor the controls had a previous cerebrovascular event (cerebral infarct, cerebral hemorrhage, or transient ischemic attack). The exclusion criteria were hemodynamically significant atherosclerotic damage of the main head arteries, stroke, Alzheimer's disease, decompensated somatic pathology.

Information on hypertension, type 2 diabetes mellitus (DM), and heart disease (coronary heart disease, myocardial infarction, valvular disease, atrial fibrillation) was based on medical history and clinical data.

Hypertension was diagnosed as systolic blood pressure > 140 mmHg. The diagnosis of hyperlipidemia was based on the guidelines established by the American Heart Association [24], and the diagnosis of type 2 DM was based on the guidelines established by the American Diabetes Association [25]. Patients with a blood glucose level > 13 mmol/L were excluded.

Alcohol drinkers were determined as individuals who drank > 1 standard drink (10 g alcohol) per week for > 2 years. Smokers were defined as individuals who smoked > 5 cigarettes per day for at least 2 years.

Neurologic impairment severity was evaluated using National Institutes of Health Stroke Scale (NIHSS) [26]. The absence of neurologic impairment according to NIHSS was evaluated as 0 points; from 1 to 6 points—neurologic impairment was determined as mild impairment; from 7 to 13 points—moderate impairment; ≥ 14 points—as significantly severe neurologic impairment, corresponding to severe stroke. Evaluation of neurologic deficit was performed on the 1 st, 3rd, 7th, and 15th days after stroke. The patient's activities of daily living were evaluated using the Barthel Index (BI). Scoring ranged from a minimal value of 0 points (total dependency of the patient) to 100 points (total independency of the patient) [27]. Evaluation of functional recovery was performed using modified Rankin scale (mRs) on the 15th day of ischemic stroke. A favorable outcome was defined as an mRs score ≤ 2. An mRs score of > 2 was used to define a poor functional outcome [28].

The nature and localization of the brain focal abnormalities were determined using MRI, including the standardized imaging protocol (T2-weighted images, T1-weighted images, fluid attenuated inversion recovery and T2*-weighted images by gradient-recall echo), and DWI modes (Siemens Magneton Verio 3.0 T) Cerebral infarction was subdivided into medium and large type. The volume that corresponded to the arterial territory in which the infarction originated was used to determine the classification. In the territory of the internal carotid artery, a large infarction is an infarction that spreads to the entire territory of one of the cerebral arteries, and a medium infarction is an

infarction that is localized within the territory of the cortical or deep branches of the middle or anterior cerebral arteries. A small infarction has a volume of up to 1.5 cm^3 and is localized in both white and gray matter.

According to MRI (DWI) data, the ischemic focus area in patients with ischemic stroke was calculated (b = 1000) dynamically (at admission, on the 3rd and 15th days), in mm^2. Measurement of irreversible changes area was performed by selection of the area "of interest" on a slice with the maximum diameter of the ischemic focus with subsequent processing of data using standard software.

Color duplex study of the brain arteries was performed according to the established procedure using Logiq 9 (GE) and iU 33 (Phillips) devices. The degree of carotid artery stenosis was determined according to the European Carotid Surgery Trialists criteria.

Transthoracic echocardiography was performed using an iU 22 (Phillips) device.

All patients with ischemic stroke obtained antihypertensive therapy. Antiplatelet therapy with acetylsalicylic acid was performed from the moment of admission. All patients received anticoagulant therapy (in case of patients with significantly evident motor impairment, for preventive treatment of thromboembolic complications) and oral anticoagulants (in case of patients with cardioembolic stroke, in 7–14 days of ischemic stroke), statins, and early rehabilitation.

2.2. Laboratory test methods

Complete blood count with determination of hemoglobin level, red blood cells (RBC), platelets (PLT), and white blood cells (WBC), and erythrocyte sedimentation rate was performed using a hematology analyzer (MEK-7222; Nihon Kohden). Main hemostasis parameters—fibrinogen level according to the Claus method, and activated partial thromboplastin time were measured using an immunoturbidimetric technique with an automatic coagulometer ACL 9000 (Instrumentation Laboratory). Hematocrit (Ht) was determined by centrifugation using Ht centrifuge (Heraeus Pico, Thermo Fisher). Tubes with 3.2% sodium citrate in a final volume ratio of 1:9 were used. The analysis was performed immediately after blood drawing.

The content of lipids and blood glucose was tested using a Konelab 30 automatic biochemical analyzer.

2.2.1. Measurement of plasma low-molecular-weight aminothiol levels

2.2.1.1. Blood samples. Control blood samples (1 mL) were obtained at 1, 3, 7, and 15 days after stroke. Venous blood was collected into tubes containing sodium citrate and centrifuged at 3000 × *g* for 3 min. The plasma for the total LMWT assay was collected, frozen at −80 °C, and stored until analysis. Plasma (100 μL) was added to 25 μL of 5-sulfosalicylic acid dihydrate solution (230 *g*/L) immediately after isolation, for *r*LMWT analysis. The samples were mixed thoroughly, frozen, and stored at −80 °C.

2.2.1.2. Biochemical assay. Total plasma LMWT levels were measured using a HPLC-UV method as described previously [29]. Reduced thiol levels were measured as described previously [30], with some modifications. Before derivatization, the samples were centrifuged for 5 min at 15,000 × *g*. We mixed 40 μL of supernatant with 40 μL of 20 mmol/L 5,5′-dithiobis-(2-nitrobenzoic acid) with 2.5 μmol/L of internal standard (penicillamine) in 0.4 mol/L Na-phosphate buffer (pH 8.0). We added 10 μL of 1 mol/L NaOH, and mixed the solution for 5 s and, and then added 12.5 μL of 1 mol/L HCl with 20 mmol/L *N*-ethylmaleimide to stop the reaction.

2.3. Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics for Windows (version 20.0). As all primary variables were not normally distributed, the data are presented in the form of median *Me*, and upper and lower quartiles. Differences between groups were tested for significance using either a non-parametric Mann–Whitney *U* test for 2 groups or Kruskal–Wallis analysis of variance for more than 2 groups and trend analysis. An analysis of the dynamics of signs in groups was carried out using Wilcoxon methods and the Friedman analysis of variance. We used a receiver operating characteristic (ROC) curve to evaluate biochemical variables when the outcome is binary (good or poor functional outcome). We performed calculation of logistic regression (0—no ischemic stroke, 1—ischemic stroke), including the control group using SPSS. We determined values of the following characteristics of the diagnostic method to determine the probability of event occurrence (ischemic stroke) for a certain case: sensitivity, specificity, prognostic value of positive result, prognostic value of negative result, and predictive accuracy. A $p < 0.05$ was considered to indicate a significant difference.

Table 1
Baseline characteristics of the patients.

Characteristics	Ischemic stroke (n = 41)	Control (n = 31)
Age, y, (median)	71.5 [59.0;78.0]	69.0[60.0;75.0]
Gender (M/F); n, (%)	15/26 (37/63)	12/19 (38.7/61.2)
Risk factors:		
Hypertension, n (%)	36 (88%)	31(100%)
Type 2 DM, n (%)	9(22%)	5(16%)
Hyperlipidemia, n (%)	12(29%)	8(26%)
CAD, n (%)	28(68%)	
Atrial fibrillation, n (%)	23(56%)	
Cigarette smoking, n (%)	19(46%)	12(39%)
Alcohol drinker, n (%)	9(22%)	6(19%)
Obesity, n (%)	11(27%)	7(23%)
Laboratory findings:		
Colesterol, mmol/L, (median)	6.0[4.9;6.8]	5.9[4.9;6.6]
TG, mmol/L, (median)	1.7[1.1;2.3][b]	1.0[0.8;1.5]
HDL-C, mmol/L, (median)	1.7[1.5;2.1]	1.9[1.4;2.6]
LDL-C, mmol/L, (median)	2.5[1.8;3.2]	2.2[1.5;3.2]
Fasting glucose, mmol/L, (median)	5.8[5.4;6.7][a]	5.0 [4.7;5.3]
Ht, %, (median)	43[41;47]	43[41;45]
aPTT,c, (median)	26.8[24.4; 28.0]	28.7[26.1;30.0]
Fibrinogen, g/L, (median)	4.5[3.8; 5.2][a]	3.58[2.97;4.06]
HGB, g/L (median)	143[118;171]	150[139;163]
RBC,10^{12}/L (median)	4.6[4.3;4.8]	5.0[4.9;5.1]
WBC,10^9/L (median)	9.6[8.5;11.0]	9.2[7.5;9.9]
PLT,10^9/L (median)	223[182;270]	218[174;253]
ESR, mm/h (median)	12[5;23]	8[5;25]

DM, diabetes mellitus; CAD, coronary artery disease; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-dencity lipoprotein cholesterol; Ht, hematocrit; aPTT, activated partial thromboplastin time; HGB, hemoglobin; RBC, red blood cells; WBC, white blood cells; PLT, platelets; ESR-erytrocyte sedimentacion rate.
Different from control ([a]P < 0,001, [b]P < 0,01).

3. Results

3.1. Baseline characteristics of the patients

Based on the baseline characteristics and laboratory data (Table 1), there were no significant differences in risk factors (hypertension, type 2 DM, hyperlipidemia, coronary artery disease, atrial fibrillation, cigarette smoking, alcohol consumption, and obesity) and in WBC count, RBC count, PLT count, and serum levels of total cholesterol, triglyceride, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol.

18 patients had large-artery atherosclerosis (44%) and 23 patients had cardioembolic stroke (56%). The total NIHSS score at admission to hospital was 7.5 (4, 17) points. The median presenting NIHSS score was 7.5 points, indicating moderate neurologic impairment. Large-artery atherosclerosis and cardioembolic stroke were diagnosed in 18 (43.9%)

and 23 (56.1%) cases, respectively. Fifteen (37%) patients had mild stroke (NIHSS < 7), 14 (34%) had moderate stroke (NIHSS 7–13), and 12 (29%) had severe stroke (NIHSS ≥14). On the 1 st day of ischemic stroke, the median of the total score according to the mRs and BI was 3 (1, 4) and 70 (20, 95), respectively. BI and mRS among patients at admission were 70 [20;95] and 3 [1;4], respectively.

According to DWI data, infarct size on the first day was 459 (178, 1454) mm^2. According to DWI data, the ischemic focus area on the 3rd day was 495 (173, 1560) mm^2. The rate of ischemic focus increase was 61%. The growth of the ischemic focus by the 3rd day of stroke (rΔSDWI1–3) was calculated as the difference ratio of the focus size on the 3rd and 1 st days and the focus size on the 1 st day of stroke: rΔSDWI1–3 = (SDWI3 – SDWI1/SDWI1) × 100%. The focus increased by 7.6%. On the 15th day, the median of infarct size was 243 mm^2 (139, 503). No statistically significant differences in infarct size between the 1 st and the 15th days were observed (p = 0.16).

3.2. Serial changes of low-molecular-weight aminothiols in blood

3.2.1. Plasma in patients with ischemic stroke

The median content of *t*, *r*, and RS of LMWTs in the blood plasma among patients compared with the controls is shown in Fig. 1. As it shows, tHcy, its reduced form and RS do not undergo significant changes in the presence of acute stroke. No changes were detected in rGSH and tCG also. The other LMWT forms demonstrated significant changes between the stroke and control groups. For instance, rCG level (and its RS) was decreased during 1–7 days and rCys level was decreased during 1–15 days after acute stroke. By contrast, tCys level was increased during 1–3 days which ultimately resulted in the decrease in CysRS. At the same time, an increase in tGSH level (1–7 days) did not lead to significant changes in its RS.

No statistically significant difference was observed between the levels of LMWTs compared with groups of patients with large-artery atherosclerosis and cardioembolic stroke (data not shown).

LMWT variables did not differ significantly according to NIHSS, BI, and mRs between groups with large-artery atherosclerosis and cardioembolic stroke.

Among the LMWTs, only *r*Cys demonstrated a statistically significant difference (Friedman non-parametric test $p = 0.04$): its level decreased from 4.8 μM to 3.8 μM during the first 3 days after stroke and then returned to its original value on the 7th and 15th days after stroke.

We found that RS of Hcy and GSH and rHcy in patients with severe neurologic deficit (NIHSS ≥14) was raised on the 3rd day after the stroke (Fig. 2a–c).

Analyzing the possible association of cerebral infarct size with LMWT levels using the Mann–Whitney *U* test, it was found that the *t*Hcy level on the 1 st day of stroke among patients with a large infarct was significantly higher compared with patients with a moderate infarct (Fig. 2d). Furthermore, the levels of tCG on the 7th day and tCys on the 3rd day in patients with small infarct were less pronounced than those

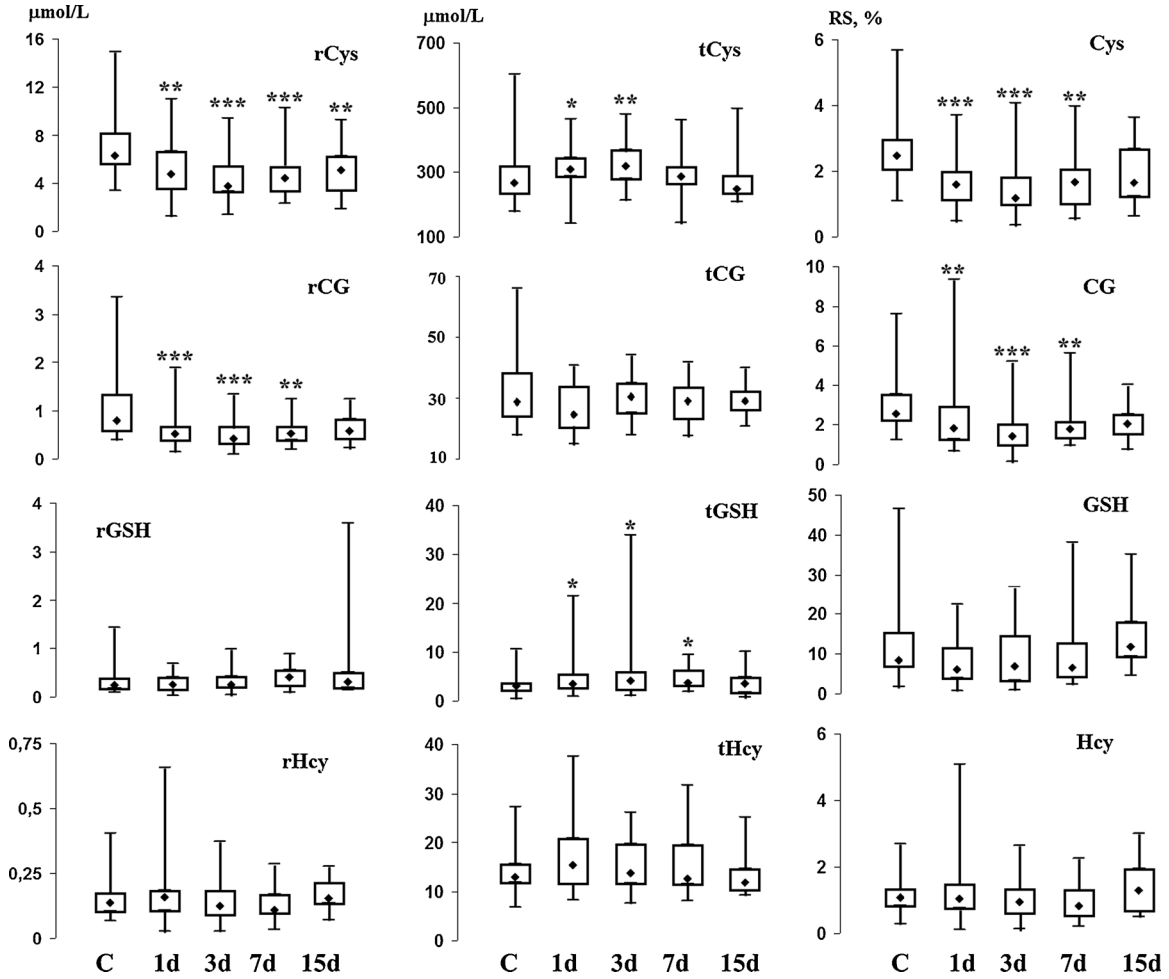

Fig. 1. Comparison of plasma low-molecular-weigt aminothiols (in μmol/L) and their redox status (in %) between acute ischemic stroke patients and controls (Mann Whitney *U* test).
C, control; CG, cysteinylglycine; Cys, cysteine;Hcy, homocysteine; GSH, Glutathione; r, reduced; RS, redox status; t, total.
* 0.01 < p ≤ 0.05, ** 0.001 < p ≤ 0.01, *** p ≤ 0.001.

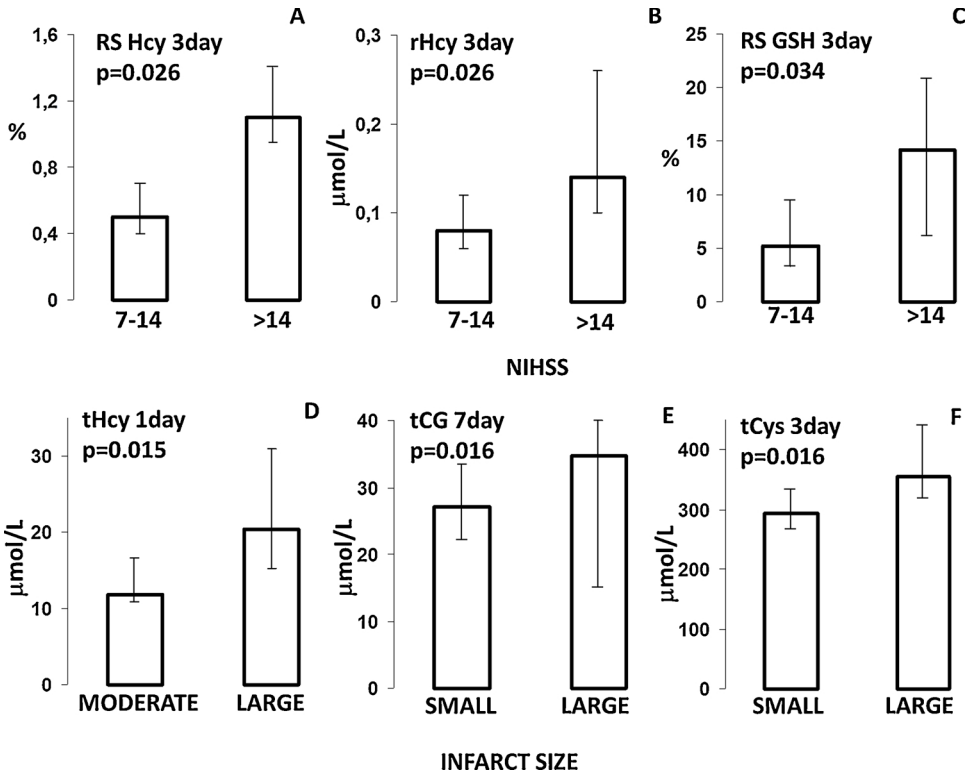

Fig. 2. A–C: The Relationship between neurological deficit and redox status of homocysteine and glutathione in patients with acute ischemic stroke (Kruskal-Wallis test). D–F: The Relationship between infarct size and aminothiol levels (Mann Whitney *U* test).
CG, cysteinylglycine; Cys, cysteine;Hcy, homocysteine; GSH, Glutathione; NIHSS, National Institutes of Health Stroke Scale; r, reduced; RS, redox status; t, total.

in patients with large infarct (Fig. 2e,f).

We used an ROC curve to evaluate biochemical variables when the outcome was binary (good or poor functional outcome). The area under the ROC curve determined that a serum level of Hcy RS > 1.6% predicts a favorable functional outcome (mRS 0–2) ($p = 0.05$) that was almost significantly different.

The use of logistic regression analysis and Wald statistics made it possible to identify 3 LMWT indices in patients with acute ischemic stroke at admission (*r*Hcy, *t*CG, and RS Cys), which with the greatest sensitivity and specificity differentiated this group from the control group. The probability of event occurrence (ischemic stroke) for an individual case is calculated according to the following formula:

$$p = \frac{1}{1 + e^{-z}}$$

where $z = 6.830 + 20.704 \cdot r\text{Hcy} - 0.134 \cdot t\text{CG} - 2.886 \cdot \text{RS Cys}$.

The corresponding results are presented in Table 2. The diagnostic sensitivity and specificity were 85.3% (68.9%, 95.1%) and 87.1% (70.2%, 96.4%), respectively. The prognostic value of a positive and negative result was 87.9% (71.8%, 96.6%) and 84.4% (67.2%, 94.7), respectively. The predictive accuracy was 86.2% (75.3%, 93.5%), and the statistical significance of the model was < 0.001.

4. Discussion

The modern concept of the resistance of brain tissue to ischemia is based on the ability of neurons to function in conditions of OS. At the present time, a completely legitimate idea of the existence of direct dependence of the disturbances of physiological processes on the state of oxidation–reduction reactions and the system of antioxidant protection has been formed.

Table 2
Cross-table of acute ischemic stroke diagnostics of control subjects and patients.

		Actual class		Per cent of correct data
		Control	Stroke	
Predicted class	Control	27	4	87.1
	Stroke	5	29	85.3
Total per cent				86.2

Brain damage leads not only to local oxidative stress, but alsoto the activation of systemic mechanisms distributing the active ROS production to peripheral vessels [5–7]. The subsequent decrease in tSH level in the blood plasma was previously demonstrated both in an experimental model of cerebral ischemia [31] and in clinical studies of acute stroke [11,13,14]. Because more than 90% of SH groups in plasma are cysteine containing protein residues, mainly albumine's Cys-34 having an abnormally low pKa ˜ 5 [32], tSH may not reflect the specific changes in rLMWTs.

Our results showed that only Cys and CysGly reduced forms and their RS undergo a significant drop during the first week after stroke. The decrease in acute ischemic stroke indicates amplification of oxidative processes, fall of Cys and CG RS, and insufficiency of antioxidant systems involved in the reduction of thiols. At the same time reduced forms of GSH, Hcy and their RS do not change significantly. However, previously it was found that rHcy level in patients with acute stroke was significantly higher than in controls [33]. This difference in the results may be due to the fact that healthy volunteers were used in [33] and patients with chronic cerebral ischemia were used as controls in the present work. Interestingly, it is mainly the decreased RS Hcy level that was associated with poor functional outcome of stroke on the border of statistical significance (Table 2). Additional investigations should be carried out in order to examine this possible correlation.

It was found previously on acute cerebral ischaemia models in rats that the reduced forms of the main LMWTs in blood plasma undergo a significant drop (by 50–90% of original value) [21,22]. The results of our work demonstrate that homeostasis disruption of LMWTs is more complex which apparently can be explained by a number of factors and individual properties influencing LMWT metabolism. Such factors are in need of further studying.

If the total decrease in rLMWT levels can be explained by OS generalization like in the case of tSH, the preservation of the RS of

individual aminothiols in these conditions indicate an activation of their production which precedes oxidation rate. GSH is the main intracellular thiol-containing antioxidant which plays a special role in providing antioxidant protection of neurons [34,35]. The observed increase in tGSH is consistent with the previously obtained results [36,37] according to which the disruption of GSH homeostasis occurs after stroke within up to 48 h. Zimmerman et al. reported an increase in tGSH serum within the first 6 h after onset, and elevated GSH peroxidase level at 1 st day after stroke [36]. Ozkul et al. noted elevated GSH serum in 70 patients within 48 h after stroke, suggesting an adaptive response to OS [37]. On the one hand, an increase in *t*Cys levels (as a progenitor and rate-limiting substrate of GSH synthesis) possibly indicates the activation of antioxidant defense in ischemic stroke. On the other hand, the rise in in *t*Cys levels may be due to an increase in glutamate level (the product of GSH hydrolysis), which not only has direct neurotoxicity but also inhibits the transport of cystine (a precursor of Cys) in neurons [35].

The active involvement of thiols in oxidation processes gave reason to consider them as potential diagnostic and/or prognostic markers of ischemic stroke. Although various different oxidation stress indicators in stroke patients have been studied, clinical data on the plasma thiol homeostasis are insufficient and controversial. Tsai et al. [11] reported that the level of tSH was significantly lower in patients with ischemic stroke than in the control group. They found a significant reduction in tSH in large-vessel disease compared with small-vessel disease groups on the 7th day after stroke. These results suggest a possible particular association between free thiol levels and stroke subtypes. They noted that lower free thiol levels in the acute period of ischemic stroke are associated with an unfavorable outcome [11]. However, serum tSH, as calculated in this study, were about 1 μmol/L or less. As a rule, this indicator is more than 200–300 μmol/L [38,39]. Leinonen et al. found a significant correlation between tSH and NIHSS scores [40]. Significant difference was also found between the tSH levels of the mild and moderate-severe NIHSS groups in [13]. However, Musumeci et al., did not confirm the correlation between NIHSS score and tSH levels [12]. The same conclusion was obtained in [14] in which the authors did not find that tSH has any effect in determining stroke severity in either type of stroke. Thus, it is not possible to draw a definite conclusion about the ability to diagnose a stroke subtype and the degree of neurological deficit using tSH.

If we now move from tSH to LMWTs, our results demonstrate that a number of LMWTs fractions, both individually and collectively, are associated with the severity of neurological deficit, infarct size, and can also be used to diagnose stroke. Our present study shows that *r*Hcy and Hcy RS on the 3rd day are higher in patients with severe neurological deficit compared with moderate deficit, suggesting that these indicators may simply reflect stroke severity. This fraction of Hcy is the most chemically active among the other LMWT forms. It was showed that it is mainly the increase in rHcy that is most associated with the development of endothelial dysfunction in experiments on human subjects [41]. At the same time, median *t*Hcy which is considered a well-known risk factor of stroke, was higher on the 1 st day for the patients with a large infarct compared with those with a moderate infarct (Mann–Whitney U test, $p = 0.015$). Similar results were obtained when comparing small and large infarct for tCys on the 3rd day and tCG on the 7th day after stroke. Although a significant correlation, though being negative, was observed between the infarct size and native tSH levels in [13], we did not find any correlation between the infarct size and rLMWTs levels.

Logistic regression analysis was performed to identify independent LMWT indicators for ischemic stroke. Wald statistics were selected to test the selection of variables for the formula. In the present study, logistic regression analysis showed that *r*Hcy, *t*CG levels, and Cys RS at admission were indicators of ischemic stroke occurrence, with a probability of 86.2%.

In this study, patients only with large-artery atherosclerosis or cardioembolic stroke were studied. The identification of LMWT homeostasis disruption in small-vessel disease in the presence of acute lacunar stroke, is of particular interest, because a strong association between the C677 T genotype of methyltetrahydrofolate reductase (a key enzyme in the regulation of the methionine cycle and homocysteine metabolism) with lacunar stroke but not with other types of ischemic stroke was found. [42]. In this regard, the role of homocysteine fractions in the activation of NADPH oxidase, which is a key mediator in the development of oxidative stress and endothelial dysfunction during lacunar stroke, remains of particular interest. [43]. However, there is still not enough data to make certain conclusions regarding the diagnostic potential of LMWT redox status and of tHcy. No differences in tHcy were found between the subtypes of ischemic stroke [44]. However, according to other data, there is reason to believe that there are differences in the association between tHcy and various small-vessel disease subtypes [45]. This is especially important in the case of the so-called. atypical lacunar syndrome, the prognosis of which is good [46]. Although it has been shown that tHcy is an independent predictor of acute lacunar infarction, this indicator should not be considered as an early prognostic marker of this disease [47].

There are several limitations to our study: the inclusion of only a small number of patients; our findings were not compared with tSH and other oxidative stress markers; the study was limited to only 15 days after the stroke and the results reported refer only to patients without lacunar cerebral infarctions (atherothrombotic or cardioembolic).

5. Conclusions

Our present study demonstrated the complexity of changes in LMWT levels in ischemic stroke, including a decrease of *r*Cys and *r*CG and their RS. An early adaptive increase of *t*GSH and *t*Cys was also observed. No differences in LMWT levels were found among stroke subtypes (large-artery atherosclerosis and cardioembolic stroke). LMWT levels may indicate the severity of neurological deficit and the size of cerebral infarction, and their determination can be of diagnostic importance both at an early stage of ischemic stroke development and during its monitoring. *r*Hcy, *t*CG levels, and Cys RS at admission were predictive factors of ischemic stroke occurrence. Our study indicates the persistency of a redox imbalance in postacute stroke-patients. Thus, an antioxidant-based therapy and monitoring of oxidative stress could be beneficial for the recovery of stroke patients, and the correction of RS imbalance in acute stroke patients may facilitate their recovery.

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