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Impact of General Anesthesia and Antioxidants on Cognitive, Static and Locomotor Functions During Laparoscopic Cholecystectomy

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Summary

Aim of the study: to reduce cognitive impairment during laparoscopic cholecystectomy by perioperative administration of drugs with antihypoxic and antioxidant effects under the control of stabilography.

Materials and methods. We studied the effect of general anesthesia and antioxidants on cognitive, static and locomotor functions during laparoscopic cholecystectomy. We studied 90 patients with acute calculous cholecystitis randomized into three experimental groups (n=30, each group). The control group was composed of 24 healthy individuals. Group 1 patients received no antioxidants, group 2 patients received the combination antioxidant drug (sodium fumarate+sodium chloride+potassium chloride+magnesium chloride) in the postoperative period, and group 3 patients received methylethylpyridinol. The patient groups were comparable in sex, age and type of inflammatory and destructive process in the gallbladder. Surgical intervention was performed under endotracheal anesthesia. Premedication with atropine and promedol was given, and anesthesia induction was carried out with propofol, fentanyl and suxamethonium. Sevoflurane, fentanyl and cisatracurium were employed to maintain anesthesia, analgesia and myorelaxation, respectively. The patients were examined before surgery, 24 and 48 hours after surgery. In the groups of patients who received antioxidant therapy, blood sampling for hematological and biochemical examinations was performed 30 min after the administration of antioxidants. Stabilographic studies and MoCA test (Montreal Scale) were performed before antioxidant administration prior to surgery and after surgery, on days 2 and 3.

Results. Neuropsychological testing revealed postoperative cognitive dysfunction on standard therapy which included impaired attention and concentration, executive function, memory, speech, visual constructional skills, abstract thinking, counting, and orientation (21 points on the MoCA scale versus 28–30 points for normal). We found that the pathogenetic factors of cognitive dysfunction included insufficient antioxidant protection, decreased TNF-α and elevated interleukin-18 levels along with an increased level of C-reactive protein in plasma, which manifested as activation of free-radical oxidation processes and reduced antioxidant system and performance of nonspecific resistance. Perioperative use of the combination antioxidant drug and methylethylpyridinol antioxidants reduced the frequency and severity of postoperative cognitive impairment in patients after laparoscopic cholecystectomy.

Conclusion. The most important pathogenetic factors of cognitive dysfunction after laparoscopic cholecystectomy include activation of free-radical oxidation, reduction of antioxidant defense system performance and lack of nonspecific resistance factors. Adding the combination antioxidant drug or methylethylpyridinol to the standard therapy reduces the intensity of radical oxygen species generation, maintains the antioxidant potential, activates production and secretion of nonspecific resistance factors, preventing the development and reducing the severity of cognitive disorders in the perioperative period. Neuropsychological testing and stabilographic examination allow identifying the risk of cognitive disorders in patients after laparoscopic cholecystectomy and provide a rationale for the use of antioxidant therapy for their prevention.

Keywords: laparoscopic cholecystectomy; cognitive disorders; free-radical oxidation; antioxidant therapy

Conflict of interest. The authors declare no conflict of interest.

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Introduction

Recent years have seen a significant increase in the incidence of cognitive disorders in various diseases, both medical and surgical ones. These disorders are manifested primarily by reduced memory and mental performance, impaired perception, processing and analyzing of information, as well as low ability to remember and retain this information [1–4]. Cognitive disorders hamper rational cognition of the world and the possibility of purposeful interaction with it [5–8]. Cognitive dysfunctions are associated with many diseases such as cerebrovascular [9], mental [10], cardiovascular [11, 12], surgical [13, 14], and infectious [15–17] ones.

The introduction of laparoscopic cholecystectomy into clinical practice has expanded the indications for the use of this method, which is considered to be the «first choice intervention» in the treatment of cholelithiasis [18-20]. Its use is associated with cognitive impairment of varying severity in up to 80% of cases [21]. Mortality in laparoscopic cholecystectomy is almost three times lower than in open cholecystectomy [22, 23]. The causes of mortality after laparoscopic cholecystectomy include myocardial infarction (0.4%), pulmonary embolism (0.3%), acute cardiovascular failure (0.4%) [24]. The nature of condition and surgical technique do not allow to fully implement proper prevention of complications and avoid the sequelae of surgical aggression. Anesthesia, along with surgical aggression, is also associated with increased risk of complications during laparoscopic cholecystectomy [25]. These factors are a major challenge for anesthesiologist requiring search for new approaches to anesthesiological support. Quite often (in 16–44% of cases) during surgical interventions, anesthesiologists make errors of varying severity such as erroneous dosing of drugs, inaccurate filling out of the anesthesia record sheet, or failure to control the changes in performance of vital organs and systems [26]. In addition, there are accumulating data on neurotoxic effects of general anesthesia resulting in the development of postoperative cognitive dysfunction, the pathogenesis of which remains poorly understood [14, 27–29]. There are few reports on the results of monitoring the cognitive functions of patients in the postoperative period, which requires further studies [25, 32].

The aim of the study was to reduce cognitive impairment during laparoscopic cholecystectomy by perioperative administration of drugs with antihypoxic and antioxidant activity under control of stabilography.

Material and Methods

Ninety patients hospitalized in Penza City Clinical Hospital № 6 named after G. A. Zakharin and the 3rd Central Military Clinical Hospital named after A. A. Vishnevsky and underwent laparoscopic surgery for acute cholecystitis with ASA severity I–III. The patients were randomized into 3 equal groups which were comparable by gender, age, types of gallbladder disease and differed only in treatment method. Group 1 patients received no antioxidant therapy during surgery, group 2 patients received a combination antioxidant drug containing sodium fumarate, sodium chloride, potassium chloride and magnesium chloride (Mafusol®) 800 ml intravenously (the volume of infusion was the same for every patient due to minor differences in body weight) during surgery, group 3 patients received antioxidant methylethylpyridinol 10 mg/kg intravenously during surgical treatment. The control group consisted of 24 healthy subjects, comparable in gender and age with the study participants.

The surgical intervention was performed under endotracheal anesthesia. Premedication was done 30 min before the patients were transferred to the operating room using 0.1% atropine 0.5 ml and 2% promedol 1 ml, and induction anesthesia was performed with propofol 2 mg/kg, fentanyl 0.01 mg/kg, and suxamethonium 2 mg/kg. After tracheal intubation, the patients were ventilated using a FabiusDraeger machine with a 1:1 air-oxygen mixture, respiratory volume of 8–10 ml/kg and respiratory rate 16–18 per minute. Anesthesia was maintained by 1.5–2‰ sevoflurane, analgesia by fentanyl 2.5–15.0 μg/kg and cisatracurium besilate was used for neuromuscular blockade.

The patients were examined before surgery, 24 and 48 hours after surgery. In the groups of patients receiving antioxidant therapy, blood sampling for hematological and biochemical tests was performed 30 min after the administration of antioxidants. Stabilographic studies and MoCA test (Montreal scale) before surgery were performed before the administration of antioxidants, while on days 2 and 3 these studies were performed 30 min after the administration of Mafusol® in group 2 patients and methylethylpyridinol in group 3 patients [33].

Blood antioxidant status was studied using the photometric method based on reaction of antioxidants (present in the sample) with exogenous hydrogen peroxide, using «Biochemmack» (Russia) kits. The serum level of proinflammatory cytokines TNF-α and IL-18 was determined by enzyme immunoassay using Vector-Best kits. C-reactive protein (CRP) content was studied by immunoturbidimetric method using «Dyakon» kits. Cognitive functions were assessed according to the Montreal scale (MoCA test). The maximum obtainable level on this scale was 30 points. Stabilographic studies were performed on a «ST-150» stabilograph using Stabip software developed by Biosoft-M (Moscow). The patient was standing upright on the stabilometer platform

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Table 1. Effect of laparoscopic cholecystectomy on changes of clinical and laboratory parameters in patients from group 1

Parameter	Value at different periods			
	Before surgery	24 hours after surgery	48 hours after surgery	Control group
Hemodynamic parameters				
Heart rate, beats per minute	87 [85; 89]*	77 [75; 80]#	77 [67; 80]#	70 [60; 80]
SBP, mm Hg	148 [145; 150]*	130 [125; 130]#	125 [120; 130]#	122 [119; 130]
DBP, mm Hg	88 [85; 90]*	78 [75; 80]#	76 [74; 80]#	76 [72; 80]
Blood neutrophil count and free radical status				
Neutrophils, $\times 10^9$/l	5.1 [4.1; 7.3]*	6.0 [4.7; 7.3]*	6.9 [5.8; 9.1]*#	3.5 [2.8; 4.2]
Spontaneous CL, units	2.6 [1.9; 4.9]	4.8 [3.0; 6.5]*#	2.5 [1.4; 4.2]	2.5 [1.4; 2.9]
Differences in CL sums, units	0.46 [–0.41; +1.33]*	–2.00 [–3.00; +0.10]*#	–2.10 [–3.09; +0.12]*#	1.30 [–0.11; +2.05]
BAS,μmol/l	299 [256; 326]	267 [196; 300]*	297 [257; 333]	306 [268; 328]
Inflammatory/proinflammatory markers				
CRP, mg/l	8.0 [3.0; 17.0]*	23.0 [15.0; 65.0]*#	41.0 [27.0; 115.0]*#	3.0 [2.5; 5.5]
TNF-α, pg/ml	2.5 [0.9; 3.3]*	3.1 [3.0; 4.0]*#	2.0 [1.8; 2.5]*	0.5 [0.4; 1.3]
IL-18, pg/ml	88.9 [51.2; 161.9]*	77.5 [45.0; 154.6]*#	68.8 [37.9; 136.5]*#	111.0 [60.0; 121.3]
Montreal scale assessment				
MoCA, points	27.0 [26.0; 28.0]	21.0 [21.0; 22.0]*#	23.0 [22.0; 25.0]*#	28.0 [27.0; 29.0]
Stabilographic parameters				
~X(OE), mm	0.80* [–3.38; 6.16]	0.67* [–4.59; 8.16]	0.93* [–4.41; 6.12]	–2.38 [–6.00; 4.18]
~X (CE), mm	0.54* [–4.78; 6.88]	2.03*# [–5.32; 8.44]	0.89*# [–5.56; 3.87]	–2.63 [–4.40; 3.53]
maxX (OE), mm	14.25* [11.40; 22.00]	15.60* [12.90; 24.10]	17.15*# [12.10; 33.90]	10.25 [9.06; 17.60]
maxX (CE), mm	13.95* [9.95; 17.50]	14.10* [12.00; 17.60]	11.70# [9.52; 16.10]	9.93 [7.40; 16.20]
DX (OE), mm	14.80* [10.40; 24.20]	18.90*# [9.97; 31.20]	16.45* [13.10; 21.90]	8.40 [6.46; 11.70]
DX (CE), mm	16.40* [8.56; 24.60]	17.70* [8.98; 22.90]	12.45# [7.35; 18.60]	10.85 [5.07; 26.05]
FX (OE), Hz	0.20 [0.14; 0.24]	0.16 [0.12; 0.22]	0.19 [0.14; 0.24]	0.20 [0.15; 0.32]
FX (CE), Hz	0.18 [0.16; 0.25]	0.20 [0.12; 0.24]	0.18 [0.14; 0.20]	0.23 [0.15; 0.29]
F60X (OE), Hz	0.50 [0.37; 0.59]	0.51 [0.37; 0.63]	0.47* [0.43; 0.63]	0.62 [0.54; 0.71]
F60X (CE), Hz	0.49 [0.35; 0.65]	0.51 [0.41; 0.57]	0.46* [0.41; 0.63]	0.60 [0.46; 0.77]
~Y (OE), mm	–30.60* [–39.30; –14.30]	–18.95*# [–35.00; –9.08]	–24.50*# [–30.30; –6.93]	–14.80 [–21.90; –9.69]
max Y (OE), mm	31.70* [21.70; 42.20]	32.75* [16.70; 42.30]	24.90*# [16.50; 34.80]	16.15 [13.75; 22.00]
max Y (CE), mm	27.00* [23.00; 36.10]	26.05* [15.50; 39.00]	25.85* [19.20; 35.50]	16.41 [12.25; 18.80]
DY(OE), mm	19.20 [16.60; 36.80]	22.15* [16.10; 36.40]	23.30*# [14.00; 28.80]	16.00 [9.90; 30.05]

Note. * — $P<0.05$ vs the control group; # — $P<0.05$ vs the preoperative values (Mann–Whitney U-test). OE — open eyes; CE — closed eyes; BAS — blood antioxidant status; SBP — systolic blood pressure; DBP — diastolic blood pressure; CL — chemiluminescence; CRP — C-reactive protein; TNF — tumor necrosis factor; IL — interleukin.

under different sensory conditions, with open and closed eyes. The duration of each test was 51.2 seconds. Stabilometry studies were performed according to the Moscow Consensus on the Use of Stabilometry and Biocontrol by Reference Reaction in Practical Medicine and Scientific Research [34].

At the preliminary stage of the study based on the stabilometric parameters in 120 patients during laparoscopic cholecystectomy a regression equation was made to estimate the compensation of antioxidant defense system:

$$P = 95 \times Fy60\% - 70 \times Fx60\% + 4V + W,$$

where P is compensation index of antioxidant protection system, Fy60% and Fx60% are quantiles of spectral power density of oscillations of pressure center frontal and sagittal planes (Hz), V is average speed of pressure center (mm/sec), W is patient weight (kg). Decompensation of the antioxidant system was assessed based on P parameter values: those less than 130 indicated significant level of decompensation, between 130 and 150 — an average decompensation, and those greater than 150 suggested good compensation of the antioxidant protection system.

Statistical analysis of the results was performed using Statistica 6.0 software package and nonparametric statistical methods. The results were considered significant at $P<0.05$. Quantitative parameters were described by median and interquartile range *Me* [LQ; HQ]. Comparative analysis of quantitative characteristics in independent groups was performed using the Kruskal–Wallis method (if $P<0.05$ the groups were considered derived from different general populations or general populations with different medians). In this case, we performed a pairwise comparison of the groups using the Mann–Whitney test. Non-parametric Wald-Wolfowitz test was used where appropriate to reveal cytokine concentrations changes. Spearman's test was used for correlation analysis of the results.

Results

The study of hemodynamic parameters revealed significantly higher HR, systolic (SBP) and diastolic (DBP) pressures in patients who did not receive antioxidant therapy before surgery vs those in the

control group. On day 2 after the surgery, these values decreased and did not differ from those in the healthy subjects (Table 1).

The neutrophil count in the peripheral blood was significantly higher compared to the control group at all stages of the study (Table 1). The highest values of spontaneous chemiluminescence (CL) parameters were observed on day 2 after surgery. The difference in CL sums test was significantly lower than in the control group on day 2 after the operation (Table 1). In view of the increase of CL values exactly on day 2, low values of the difference in CL sums test may indicate reduced activation reserve when neutrophils were exposed to endogenous substances.

The serum CRP level in group 1 patients was significantly higher at all stages of the study compared to similar parameters in healthy subjects (Table 1). The TNF-α level before surgery was higher compared to controls (P=0.04, Wald-Wolfowitz test). On days 2 and 3, TNF-α levels were also significantly higher (P<0,05, Mann–Whitney test). Preoperative and 3-day postoperative concentration values of another proinflammatory cytokine IL-18 were lower compared with controls (P=0.03 and P=0.003, respectively, Wald-Wolfowitz test).

The values of blood antioxidant status (BAS) parameters significantly decreased 24 hours after laparoscopic cholecystectomy and were less than the control values. The preoperative examination revealed a positive correlation (R=0.64; P=0.0001) between the severity of spontaneous CL and CRP level, which could indicate a systemic inflammatory response, and a negative correlation between CL and antioxidant activity (R=–0.69, P=0.0006), which suggests inhibition of antioxidant enzymes with increased free-radical oxidation processes and increased levels of free oxygen radicals.

The use of the MoCA test was the «gold standard» for diagnosing cognitive dysfunction. There was a significant decrease in MoCA test values 24 hours after surgery. Both 24 and 48 hours after surgery, the MoCA test values were significantly lower than those before surgery vs the control group (Table 1).

A significant correlation between the MoCA and BAS values (R=0.46; P=0.03) was revealed 48 hours after surgery. This confirms the involvement of free-radical oxidation products in the pathogenesis of cognitive disorders.

Prior to surgery, there was a significant shift of the center of pressure (COP) vs the control relative to the Y axis in the «open eyes» (OE) and «closed eyes» (CE) positions (Table 1). On day 2, there was a significant shift of ~Y(OE) and ~Y(CE) to the positive direction, resulting in the position of the COP (OE) not differing from the control values, which could indicate a decrease in pain in the patients. We also found significant differences of the stabilographic indices at all stages of the study with the control group: increased values of max Y(OE), max Y(CE), and DX(OE), increased maximum amplitude of COP on the X axis (OE) on days 2 and 3 vs the control group. The described changes may indicate a complex combined impact of acute cholecystitis, surgical trauma and general anesthesia on the patients.

A significant increase in the statokinesiogram area S (OE) 24 hours after surgery was revealed vs the controls (323.0 mm^2) and its value before surgery (230.0 mm^2); 48 hours later, these parameters decreased, but remained higher than prior to the surgery. The S (CE) parameter on day 2 did not differ from the control values, though there was a tendency of its increase both on days 2 and 3 after the surgery. On day 2, a significant correlation between the MoCA values and the statokinesiogram (OE) area (R=–0.48; P=0.02), as well as between MoCA score and Romberg's coefficient (R=0.45; P=0.03) was revealed. The LFS (CE) on day 2 after surgery was significantly lower than the preoperative values and those of the controls. On day 3, there was a correlation between CL and LFS (OE) parameters (R=–0.4; P=0.03), indicating the relationship between these parameters and free-radical oxidation.

Forty-eight hours after surgery, the following values were also found to be significantly higher than those in the control group: maxX (OE); maxY (OE) and (CE) and DX(OE) (Table 1). On day 3 we found a correlation between CL and LFS (OE) values (R=–0.4; P=0.03), indicating an increase in statokinesiogram area and a decrease in LFS with increased free-radical oxidation. Values of total antioxidant activity correlated with values of 60% energy relative to the F60% Y (OE) sagittal area (R=–0.53; P=0.02) and the frequency of oscillations along the Y axis (FY(OE) (R=–0.57; P=0.01). This correlation shows the correspondence of larger values of BAS with lower values of 60% of the energy of the oscillation spectrum and the frequency of oscillations on the Y-axis. Twenty-four hours after surgery, there was a 1.75-fold increase in the number of patients with severe decompensation of antioxidant defense and a 2-fold decrease in the number of patients with moderate decompensation, while the number of patients with P values over 150 remained practically at the same level.

Group 2 patients who received Mafusol® after surgery demonstrated significantly higher HR, SBP and DBP compared to controls (Table 2) and a correlation between HR and CRP level (R=0.69; P=0.0006) in the preoperative period. We detected a higher neutrophil count before surgery, on days 2 and 3 after surgical intervention vs controls. Values of spontaneous chemiluminescence and difference in CL sums test did not differ from control values at all stages of the study [34, 35]. Values of BAS in pa-

Table 2. The effect of Mafusol® (group 2) and methylethylpyridinol (group 3) on hemodynamic parameters in the postoperative period, Me [LQ; HQ].

Parameter	Value at different periods			
	Before surgery	24 hours post surgery	48 hours post surgery	Control group
Heart rate, bpm, G2	87 [85; 88]*	77 [69; 80]*#	78 [75; 80]#	70 [60; 80]
Heart rate, bpm, G3	84 [80; 90]*	77 [70; 80]#	77 [72; 80]#	
Systolic BP, mm Hg, G2	149 [146; 151]*	130 [125; 130]#	125 [120; 130]#	122 [119; 130]
Systolic BP, mm Hg, G3	147 [145; 148]*	130 [130; 135]#	130 [125; 130]#	
Diastolic BP, mm Hg, G2	89 [86; 90]*	75 [70; 80]#	76 [70; 80]#	76 [72; 80]
Diastolic BP, mm Hg, G3	87 [80; 90]*	130 [130; 135]#	76 [72; 80]	

Note. G2 — group 2; G3 — group 3. * — $P<0.05$ vs the control group; # — $P<0.05$ vs the preoperative values (Mann–Whitney *U*-test).

tients treated with Mafusol® did not differ from those in the control group, but nevertheless on day 2 after the surgery a significant decrease of this parameter was found ($P=0.01$).

The serum TNF-α content before surgery, 24 hours and 48 hours after surgery was lower compared to controls ($P=0.03$). The decrease in the concentration of this cytokine in the blood serum may be due to the detoxifying effect of Mafusol®. The IL-18 levels did not differ significantly from the control and postoperative values.

On days 2 and 3 of the postoperative period there was a dramatic increase in serum CRP level vs the control ($P<0.05$). Twenty-four hours after surgery we revealed a correlation between CRP and BAS ($R=–0.39$; $P=0.04$). Correlation analysis also showed a correlation between spontaneous CL and the difference in CL sums before surgery ($R=–0.82$; $P=0.0001$) and 24 hours after surgery ($R=–0.51$; $P=0.02$).

The MoCA scale scores before surgery, 24 and 48 hours after surgery were significantly lower than those of the controls. Meanwhile, the lowest values of the MoCA scale (24.0 points) were registered in the patients 24 hours after laparoscopic surgery. However, the difference in the mean values before and one day after surgery was insignificant, which does not indicate cognitive impairment in group 2 patients.

Stabilography data revealed a significant shift of COP (OE) on the Y axis to the negative direction, a trend to shift of ~Y(OE) to the positive direction on day 2, resulting in the position of COP on Y axis not differing from the values in the control group. We also identified parameters which were different from the control values at all stages of the study. They were max Y(OE), max Y(CE), L (OE), V (OE), Ei (OE). On days 2 and 3, we observed significant increase in maximal COP amplitude on the X (OE) axis compared to the control. On day 2, a negative correlation between the MoCA parameters and the statokinesiogram area ($R=–0.44$; $P=0.03$) was revealed only in the CE position. Significant changes when comparing the *P* parameter values with the control were recorded only prior to surgery. There was a positive correlation between the antioxidant activity compensation and the difference in CL sum in patients 24 hours after surgery ($R=0.52$; $P=0.02$), which, in turn, had a steady negative correlation with the values of spontaneous CL.

Group 3 patients had significantly higher HR, SBP, DBP compared to the control group before the surgery, but there was a decrease of HR and BP on day 2 (Table 2). Thus, although initially in all groups of patients, hemodynamic parameters differed significantly from the ones of healthy controls, during the treatment they «levelled out», which suggests the comparability of the studied groups. In addition, we revealed a higher blood neutrophil count in patients at all stages of the study. Values of differences in CL sums test and luminol-dependent CL did not differ from the control at all stages of study in the group 3 patients. Differences in BAS values vs the controls were observed only before the surgery ($P=0.04$), while no significant differences of this parameter with the control were observed during the study.

The serum TNF-α level at all stages of the study was lower than the control values, which could be due to the antitoxic effect of methylethylpyridinol. The serum CRP level before surgery did not differ from the control but increased on days 2 and 3 after surgery. A negative correlation between CRP and BAS ($R=–0.55$; $P=0.02$) was seen 24 hours after surgery. In addition, a significant negative correlation between spontaneous CL and difference in CL sums was found at all stages of the study: before surgery, $R=–0.78$ ($P=0.001$); 24 hours after surgery, $R=–0.49$ ($P=0.02$); 48 hours after surgery, $R=–0.6$ ($P=0.001$).

A comparative frequency analysis of the patient distribution depending on P parameter grading showed that in Groups 2 and 3 on day 2 after surgery no increase in the number of patients with P level less than 130 was seen, unlike group 1, where the number of such patients increased 1.75 times. In the patients who received antioxidants perioperatively we didn't observe a decrease in the antioxidant defense compensation, unlike those from group 1. The fact that patients had a high compensatory ac-

tivity of the antioxidant system while having a high reactivity reserve of neutrophils can be considered another significant result of the study. In group 3 patients who received methylethylpyridinol after surgery, the postoperative values of antioxidant activity did not differ from those of the control group.

Discussion

Thus, the neuropsychological testing indicated that patients with laparoscopic cholecystectomy who received standard treatment had such postoperative cognitive disorders as impaired attention, executive function, memory, speech, visual and constructive skills, abstract thinking, counting, and orientation. The mean MoCA score one day after surgery was 21 points, differing significantly from both the preoperative score of 27 points (P=0.01) and the mean score of healthy subjects of 28 points (P=0.01). Forty-eight hours after surgery, the mean MoCA score increased to as low as 23 points.

For patients in groups 2 and 3 who received antioxidant therapy, the mean scores on the MoCA scale, even on day 1 after surgery, were 26 and 27, respectively, and did not differ significantly from both the preoperative and healthy subjects scores. This suggests that antioxidant therapy reduces the severity and frequency of cognitive impairment during laparoscopic cholecystectomy.

The results of biochemical and immunological tests indicate that the main pathogenetic factors of cognitive disorders include insufficient antioxidant defense system, decreased TNF-α and increased IL-18 levels along with the increased serum level of C-reactive protein, which manifests in activation of free-radical oxidation, reduced antioxidant system performance and nonspecific resistance of the body. Our results are in line with the previous findings [36].

Conclusion

We found a relationship between stabilographic parameters and indicators of cognitive functions, biochemical parameters characterizing the blood antioxidant status, which offers broad prospects for their use in preoperative preparation of patients, risk assessment of cognitive disorders and decision-making on the prescription of antioxidant therapy.

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Use of a Neurometabolism-Targeting Drug in Prevention of Postoperative Cognitive Dysfunction

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Summary

Aim of the study: to evaluate the feasibility of preventing cognitive dysfunction after long-term surgery in elderly patients using an original neurometabolic succinate-containing drug.

Material and methods. A multicenter, double-blind, placebo-controlled randomized trial enrolled 200 patients aged 60–80 years who underwent elective cardiac or orthopedic surgery. The patients received either the study drug (inosine + nicotinamide + riboflavin + succinate) (treatment group, *n*=101) or a placebo (control group, *n*=99) intravenously for 7 days then orally for 25 days. Efficacy was assessed by the change in the Montreal Cognitive Assessment Scale (MoCA) score at the end of the treatment course compared with the preoperative level.

Results. Before surgery, the total MoCA score values did not differ between the groups. By the end of the treatment course (31 days after surgery), the MoCA total score was 26.4±1.96 in the main group and 25.0±2.83 in the control group (P<0.001). The intergroup difference in the mean change in the MoCA total score on day 31 was 1.56 points (95% CI 1.015; 2.113; P<0.0001) favoring the study drug in all randomized population. The lower limit of CI (1.015) exceeded the limit of superiority set by the protocol (0.97 points), which allowed acceptance of the hypothesis of superiority of the study drug over placebo with respect to the primary efficacy criterion. No significant differences in the frequency of adverse events were found between the groups.

Conclusion. The succinate-containing study drug demonstrated an acceptable safety profile and helped to reduce the severity of postoperative cognitive dysfunction in elderly patients who underwent a major surgery, which allows recommending the drug for prevention of postoperative cognitive impairment in high-risk patients.

Keywords: succinate-containing drugs; succinic acid; prevention of postoperative cognitive dysfunction

Identifiers. NCT03849664 Unique Protocol ID: CYT-COG-16

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Conflict of interest. The authors declare no conflict of interest. The OOO NTFF POLYSAN company had no influence on the study design, analysis of the material obtained, interpretation of the results, and preparation of the paper.

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Introduction

Postoperative cognitive dysfunction (POCD) manifests as impaired higher cortical functions and difficulty concentrating, which entail learning, performance, mood, and sometimes self-care problems. The cognitive impairment in the postoperative period can worsen the outcome, increase the length of stay in ICU and inpatient treatment, and reduce the quality of life of patients and their families, including in the long term after surgery [1]. POCD is a risk factor for mortality: its persistence 3 months after surgery correlates with an increased risk of death in the following 8 years [2].

Postoperative cognitive disorders occur more frequently in elderly patients undergoing major surgery. The prevalence of these conditions among elderly patients reaches 48–65% in abdominal, thoracic, orthopedic, and vascular surgical practice [3]. Age is a more significant risk factor than the type of surgery, but the issue of POCD is particularly relevant in cardiac and orthopedic surgery: the average incidence of this complication after cardiac surgery is 48–51% within the first 3 weeks, 21–26% within 6 months, 15–24% within a year, and, according to various data, from 24% to 42% within a year and beyond [4]. There is no single factor responsible for the development of POCD; randomized studies have shown that the use of a cardiopulmonary bypass during surgery [5] and the choice of anesthesia method [6] have no significant effect on the incidence of postoperative cognitive impairment. Apart from the patient's age, low level of education and preexisting cognitive deficit (in particular, associated with central nervous system diseases) predispose to this condition [7].

Despite the clinical and social impact of the problem, there are currently no drug regimens for the prevention or treatment of POCD with proven efficacy. A promising trend is the use of drugs with neuroprotective and neurotrophic effect. The concept of the neurocognitive reserve, which is defined as an active ability of the brain to effectively resist damaging factors, compensating for cognitive deterioration caused by various diseases, has been developed [8]. The potential capacity of strategy to increase cognitive reserve using chemical agents targeting neurometabolism was demonstrated in small groups of patients at high risk of developing postoperative cognitive impairment [9].

One of the drugs that may target neurometabolism has been tested during cardiac surgery (Cytoflavin®, OOO NTFF POLYSAN, Russia). The positive effect of this succinate-containing drug on cognitive functions has been revealed in patients undergoing cardiac surgery with cardiopulmonary bypass and on the beating heart. The use of the drug early after cardiac surgery reduced the likelihood and severity of cognitive, speech and attention impairment, promoted recovery of spontaneous activity, memory, emotional behavior, voluntary acts, and resulted in a rapid return of patients to active life [10, 11].

The aim of the study was to evaluate the feasibility of preventing cognitive disorders after long-term surgery in elderly patients using an original neurometabolism-targeting succinate-containing drug.

Material and Methods

A multicenter double-blind placebo-controlled randomized trial was conducted under the supervision of the Ethics Council of the Russian Federation Ministry of Health in accordance with the ethical principles outlined in the Declaration of Helsinki of the World Medical Association (Fortaleza, 2013) and the regulatory documents in force in the Russian Federation. The study protocol CYT-cog-16 (clinicaltrials.gov: NCT03849664) was followed at 13 research centers in the Russian Federation from February 13, 2018 (screening of the first patient) until November 29, 2019 (date of closure of the last center). The study was sponsored by NTFF POLYSAN.

Men and women 60–80 years old who signed informed consent to participate in the study and were scheduled to undergo cardiac surgery without cardiopulmonary bypass or orthopedic surgery (hip arthroplasty, osteosynthesis for fractures of the proximal third of the femur, etc.) under general or combined anesthesia were included in the study. The other criteria for enrollment included mental capacity, absence of severe cognitive impairment (Montreal Cognitive Assessment (MoCA) score ⩾17 [12], Mini Mental State Examination (MMSE) score ⩾19 [13]), lack of reproductive potential or consent to use adequate contraceptive methods.

The non-inclusion criteria were intolerance to the components of the study drug; emergency character of surgery; repeated surgery; ASA anesthesia risk level ⩾5 [14]; severe visual and hearing impairments that prevent the performance of neuropsychological tests; surgery under general anesthesia in the previous 3 months; decompensated renal or hepatic failure; chronic obstructive pulmonary disease, diabetes mellitus; terminal chronic incurable disease; history of cancer, psychiatric diseases, HIV infection, syphilis, tuberculosis, alcohol, drug or medication abuse, consumption of 5 or more units of alcohol per week; documented psychiatric or neurodegenerative disease; continuous use of psychotropic drugs; use of nootropic drugs within the previous 3 months; communication,

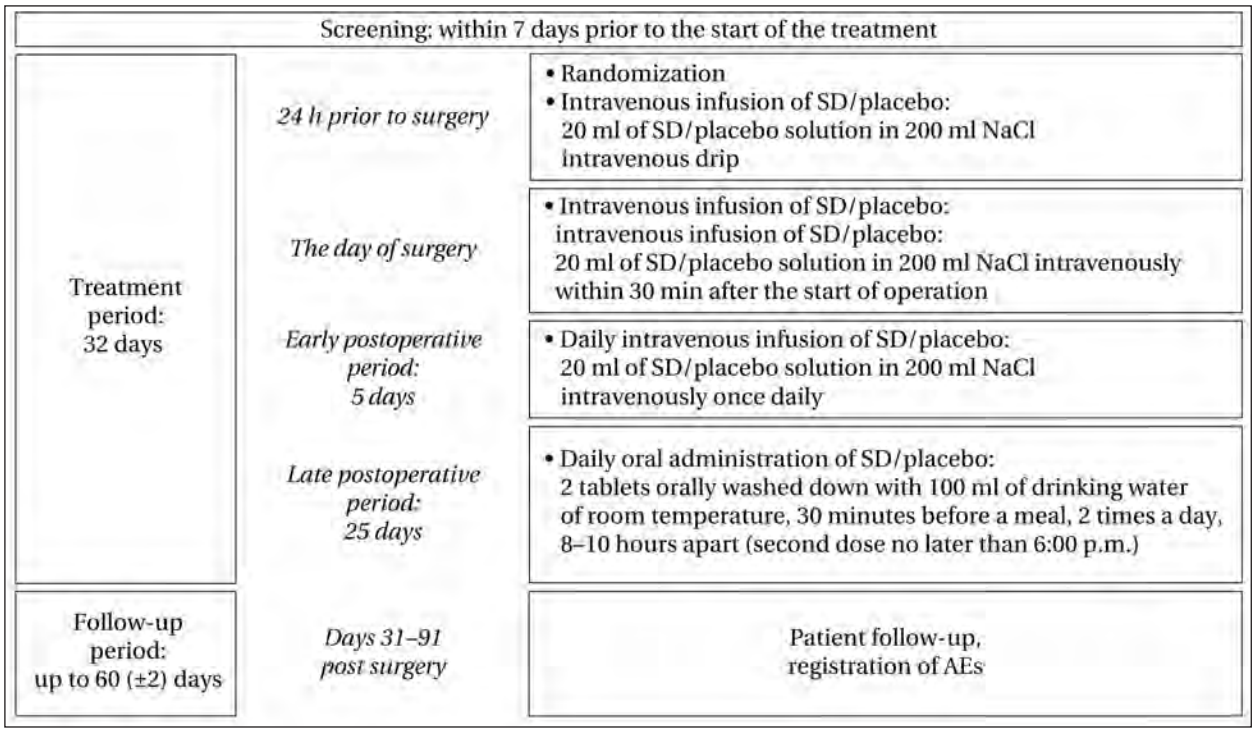

Fig. 1. The flowchart of the study.
Note. SD — studied drug; AEs — adverse effects.

sensory, motor, or any other deficit that prevents the patient from complying with the study protocol; history of any other significant condition preventing study participation (according to the investigator's opinion).

The patients were allocated to the main group, which included those receiving the study drug Cytoflavin® (succinate + nicotinamide + inosine + riboflavin, manufactured by LLC NTFP «POLYSAN», Russia) according to the chart in Fig. 1, and the control group who received placebo according to the same scheme by 1:1 block randomization.

The primary efficacy endpoint was the change in the MoCA score [12] by the end of the treatment course (day 31 days post surgery) versus the preoperative level. The MoCA score was assessed before surgery, at the end of the treatment period, and at the end of the follow-up period. The forms with different task options were used to avoid the memorization effect.

Secondary efficacy endpoints included change in the MMSE cognitive status score [13] at the end of the treatment course (day 31 post surgery) and at the end of the follow-up period (day 91 post surgery) compared to preoperative levels; change in the MoCA score at the end of the follow-up period compared to preoperative levels; proportion of patients who developed postoperative delirium during the first 96 h after surgery; length of ICU and hospital stay; change in the total score on the European Quality of Life Questionnaire (EQ-5D) at the end of the follow-up period compared to baseline values; the percentage of postoperative deaths; the percentage of patients with reduced scores on two and more neuropsychological tests by more than 20% of baseline values at the end of the treatment course and at the end of the follow-up period.

The neuropsychological test battery [15] included the TMT test A, the 10 word recall test, the Schulte table, the Wechsler memory scale, as well as the MoCA and MMSE scales, for which a decrease of more than 20% from the initial score was considered significant. Psychodiagnostic assessment was performed by a psychologist with appropriate professional qualifications. Preoperative risks were assessed using ASA scales, delirium risk assessment scales in general surgery [16] and cardiac surgery [17], fragility index [18]. Blood loss volume and hematocrit value, delirium development according to CAM-ICU (Confusion Assessment Method Intensive Care Unit) scale [19], severity of postoperative pain according to visual analogue scale, episodes of clinically significant BP changes and blood oxygen saturation (SpO_2) <90%, depression and anxiety according to Hospital Anxiety and Depression Scale (HADS) [20] were registered in early postoperative period; in the late postoperative period, the degree of dependence in the performance of daily functions according to the Katz index was assessed [21].

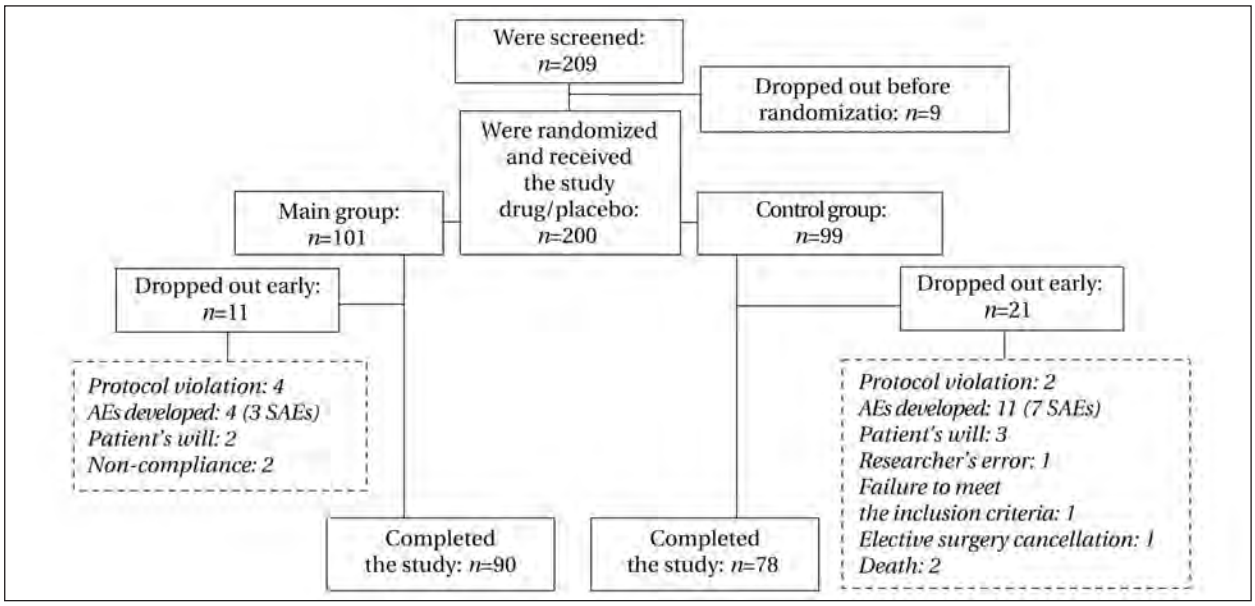

Figure 2. Flow diagram of subject recruitment and retention in the CYT-cog-16 clinical trial.
Note. AE — adverse effect; SAE — serious adverse effect.

Safety analysis was performed based on the frequency of adverse events (AEs), serious adverse events (SAEs); abnormal vital signs (BP, HR, respiratory rate, body temperature, SpO_2), laboratory parameters, ECG findings were recorded as AEs. Coding of AEs and preexisting diseases/comorbidities was performed using Medical Dictionary for Regulatory Activities (MedDRA, version 22.1), coding of previous and concomitant treatments was performed using the ATC classification.

SAS 9.4 software (SAS Institute Inc, USA) was used for statistical analysis. For qualitative variables, comparisons between groups were made using Pearson's χ^2 test or Fisher's exact test. For quantitative variables subject to normal distribution, comparison between groups was performed using Student's *t*-test or Student's *t*-test for dependent samples; for quantitative variables with non-normal distribution, Mann–Whitney *U*-test (for independent samples) or Wilcoxon *T*-test (for dependent samples) were applied. The Shapiro–Wilk test was used to verify the normality of the data distribution. The changes in the variables were assessed using the mixed-model analysis of variance with repeated measures (ANOVA MMRM). Differences were considered significant at $P<0.05$.

To assess the primary efficacy endpoint, we calculated the 95% confidence interval (95% CI) for the differences in the group mean values of the individual MoCA score changes at the end of the treatment compared with the preoperative level. To prove the hypothesis of superiority of the study drug compared with placebo, the lower limit of the 95% CI for the difference in the group mean values of individual changes was required to be higher than the limit of superiority of 0.97. To test the hypothesis of superiority of the study drug over the comparison drug at a significance level of 0.05 (5%) and with a power of 0.8 (80%), we calculated the minimum number of randomized patients who would complete the study per protocol to be 98 (49 patients per group). Given the high probability of patient dropout during the study, we planned to randomize at least 200 patients.

To study efficacy criteria in individual patient subpopulations, given the large number of subpopulations of interest and the large number of studied efficacy criteria, we chose the decision tree method, which allowed us to evaluate the potential effect of all factors of interest on the efficacy of the studied treatment, omitting the missing values.

Results

We screened 209 patients, randomized 200 patients who received at least one dose of study drug/placebo, and composed the ITT (Intent-To-Treat — all randomized patients who received at least one dose of study drug) population; 168 patients who completed the study according to protocol were included in the per-protocol (PP) population (Fig. 2).

The study groups were comparable in terms of demographic characteristics, comorbidities, med-

Table 1. Baseline patient characteristics.

Parameter	Values in groups		*P* value
	Main, *n*=101	Placebo, *n*=99	
Age, years (*M*±*SD*; *Me* [Q25; Q75])	68±5.23 67 [65; 71]	68±5.8 66 [63; 72]	0.949
Female sex, *n* (%)	66 (65.3)	55 (55.6)	0.157
Body mass index (*M*±*SD*; *Me* [Q25; Q75])	29.3±4.42 28.9 [26.4; 32.2]	28.7±4.85 28.0 [25.7; 32.2]	0.362
Any comorbidity, n (%)	100 (99.01)	98 (98.99)	>0.999
Cardiac disorders	90 (89.1)	92 (92.9)	0.460
Vascular disorders	33 (32.7)	24 (24.2)	0.212
Nervous system disorders	27 (26.7)	24 (24.2)	0.747
Any treatment within the previous 30 days	77 (76.2)	77 (77.8)	0.796
Preoperative ASA score	Class II 31 (30.7) Class III 63 (62.4) Class IV 7 (6.9)	Class II 26 (26.3) Class III 65 (65.7) Class IV 8 (8.1)	0.772
Surgical Risk Scale, mean points (±SD; n; *Me* [Q25; Q75])	0.9 (±0.83; 74) 1 [0; 2]	1.3 (±1.01; 70) 1 [0; 2]	0.042
Delirium risk assessment in cardiac surgery, mean points (±SD; n; *Me* [Q25; Q75])	0.4 (±0.69; 27) 0 [0; 1]	0.4 (±0.63; 29) 0 [0; 1]	0.681
Frailty index, mean points (±SD; n; *Me* [Q25; Q75])	0.9 (±0.83; 101) 1 [0; 1]	1.1 (±0.81; 99) 1 [0; 2]	0.265

Note. *M*±*SD* — arithmetic mean ± standard deviation; *Me* [Q25; Q75] — median [lower quartile; upper quartile]; *n* — number of subjects in the group.

Table 2. Distribution of patients according to the surgical intervention type.

Parameter	Values in groups		*P* value
	Main, *n*=101	Placebo, *n*=99	
Elective surgery performed, *n* (%)	99 (98.0)	96 (97.0)	0.982
Cardiac surgery	26 (25.7)	28 (28.3)	0.686
Orthopedic surgery	73 (72.3)	68 (68.7)	0.578
Endoprosthetic reconstruction	66 (65.3)	59 (59.6)	0.678
Osteosynthesis	6 (5.9)	9 (9.1)	0.489
Other	1 (1.0)	0 (0.0)	0.972

Note. *n* — number of subjects in the group.

ical history and the frequency of concomitant treatment, as well as the general anesthesia risk class (Table 1).

Elective surgery was performed on 99 patients in the main group and 96 patients in the placebo group (Table 2); 2 and 3 patients, respectively, dropped out of the study prematurely in the preoperative period.

Patient compliance at the stage of intravenous infusion of the SD/placebo solution was 100% (SD/placebo was administered by medical professionals); further on, in both study groups, the average compliance was above 80%.

The results of the analysis of the MoCA score and its change at the end of the treatment course (day 31) and the follow-up period (day 91) compared with the preoperative level are presented in Table 3. The difference between the SD group and the placebo group in terms of mean change in the total MoCA score at day 31 was 1.564 points (95% CI 1.015; 2.113). Thus, the lower limit of this CI (1.015) exceeded the limit of superiority set by the protocol (0.97 points). Based on the above results, the superiority of Cytoflavin® SD over placebo was confirmed with regard to the primary efficacy endpoint in the study populations. At the end of the treatment period (day 31), the groups also differed significantly in the absolute value of the total MoCA score in favor of the study drug.

When analyzing the changes in the total score on the MoCA scale (ANOVA MMRM) at the end of the treatment course (day 31) and at the end of the follow-up period (day 91) compared with the preoperative level with the inclusion of age group (younger than 70 years/70 years or older) and type of surgery (cardiac surgery/orthopedic surgery) cofactors, we found that these had no significant effect on achieving the primary efficacy endpoints.

On day 91 post surgery, the changes in the MMSE score versus the preoperative level significantly differed between groups in favor of the study drug and were 1.2±2.06 (1; [0; 2]) in the main group and 0.7±2.26 (1; [0; 2]) in the placebo group (*P*=0.0027, ANOVA MMRM), we also observed a small difference between the groups in terms of

Table 3. Group mean values of MoCA scores and their changes by the end of the treatment and follow-up periods.

MoCA score, points	Values in the groups				*P* value
	Mean, *n*=101		Placebo, *n*=99		
	M±*SD*	*Me* [Q25; Q75]	*M*±*SD*	*Me* [Q25; Q75]	
ИBaseline total	24.9±2.76	25 [23; 27]	24.9±2.63	25 [23; 27]	0.941
On day 31	26.4±1.96	27 [25; 28]	25.0±2.83	25 [24; 27]	<0.001
On day 91	26.6±1.81	27 [25; 28]	26.3±1.69	26 [25; 27]	0.299
Change in total score vs preoperative value					
On day 31	1.7±2.4	2 [0; 3]	–0.1±2.5	1 [–1; 2]	<0.001
On day 91	1.9±2.60	2 [0; 4]	1.2±2.02	1 [0; 2]	0.121
Difference in MoCA score change between patients in the main group and the placebo group:					
in ITT population	1.564 points			(95% CI 1.015; 2.113)	
in PP population	1.556 points			(95% CI 1.005; 2.106)	

Note. *M*±*SD* — arithmetic mean ± standard deviation; *Me* [Q25; Q75] — median [lower quartile; upper quartile]; ITT — intent-to-treat (all randomized patients who received at least one dose of the study drug); PP — per protocol population (all randomized patients who completed the study without protocol violations).

Table 4. Summary table of adverse events and serious adverse events.

Parameter	Values in groups		*P* value
	Main, *n*=101	Placebo, *n*=99	
Any AEs (including SAEs), *n* (%), number of AEs	63 (62.40%), 151	67 (67.68%), 156	0.433
AEs with no criteria for severity, *n* (%), number of AEs	59 (58.42%), 144	57 (57.58%), 141	>0.999
SAEs, *n* (%), number of AEs	4 (3.96%), 7	10 (10.10%), 15	0.103

Note. n — number of subjects in the group; AE — adverse event; SAE — serious adverse event.

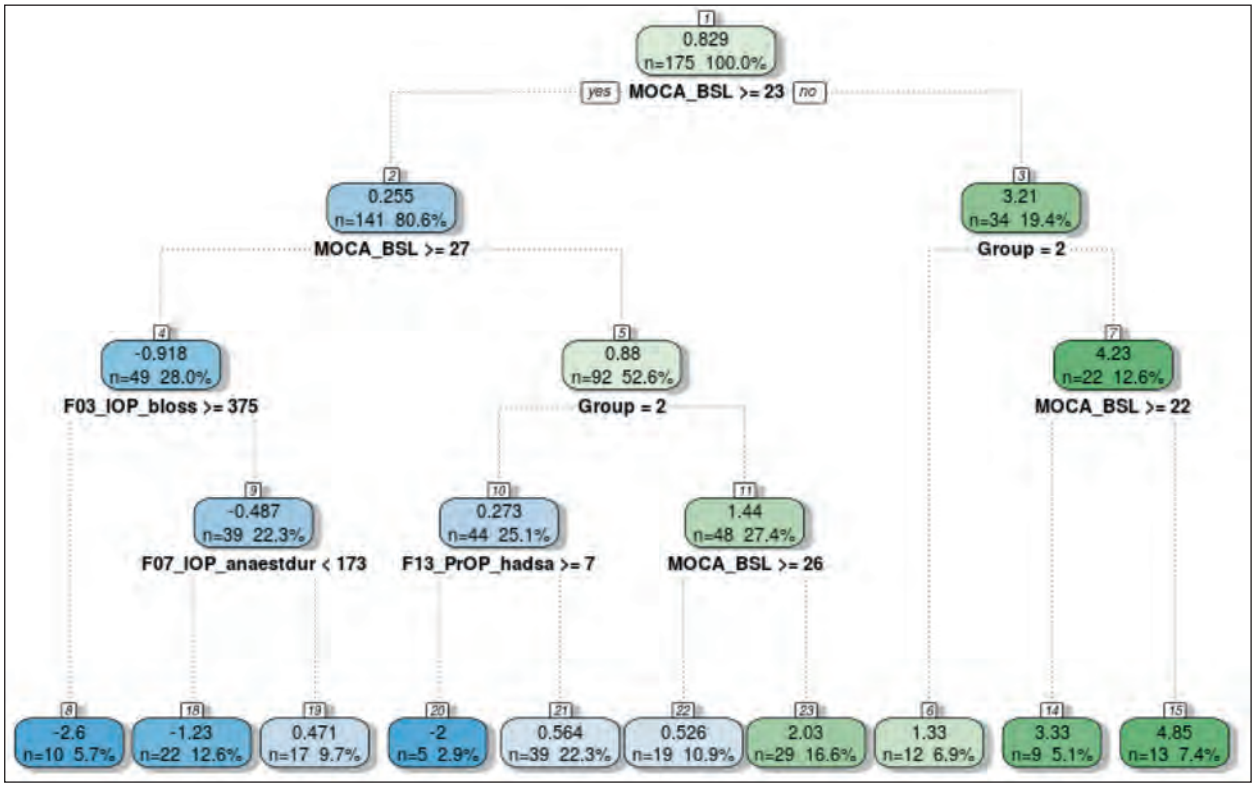

Fig. 3. Decision tree for the primary efficacy endpoint. Changes in the MoCA score by the end of the treatment period.
Note. MOCA_BSL — baseline MoCA score; F03_IOP_bloss — intraoperative blood loss; F07_IOP_anaestdur — duration of anesthesia; F13_PrOP_hadsa — preoperative HADS (Hospital Anxiety and Depression Scale) assessment.

the mean group MMSE score in favor of the study drug, 28.8±1.34 (29; [28; 30]) in the main group and 28.0±1.92 (28; [27; 30]) in the placebo group (P=0.003, ANOVA). At the end of the treatment course (day 31 post surgery), the percentage of patients with a reduction of more than 20% from baseline in two or more neuropsychological tests was lower in the Cytoflavin® group, 6.9% (7/101) of patients versus 16.2% (16/99) in the placebo group (P=0.041). At the end of the follow-up period (day 91), this proportion was 4.0% (4/101) and 9.1% (9/99) in the Cytoflavin® and placebo groups, respectively; the differences between the groups did not reach significance though (P=0.141). The odds ratio for «no worsening on two or more neuropsychological tests» was 2.5886 (95% CI for OR 1.0153; 6.6001) at Visit 9 and 2.4250 (95% CI for OR 0.7215; 8.1503) at Visit 10. For other secondary efficacy endpoints, no significant differences were found between the study groups. Postoperative delirium did not occur in all patients in both groups at each assessment point.

Analysis of the effect of various potential factors on the achievement of primary and secondary efficacy endpoints using the decision tree method revealed no significant effects of the study drug/placebo on the rate of intraoperative episodes of clinically significant hypotension and decreased oxygen saturation (SpO_2<90%), intraoperative blood loss, duration of anesthetic care, number of episodes of clinically significant BP changes (which required additional antihypertensive treatment) during ICU monitoring, postoperative hematocrits level, as well as the preoperative delirium risk scores, postoperative pain severity, anxiety, depression, and dependence in daily activities. At the same time, the MoCA baseline score before the start of therapy had the highest significance with respect to MoCA scores: patients with more severe (<23 points) cognitive impairment at the study start (n=34, 19.4%) had greater MoCA score changes at the end of the treatment period (day 31) — 3.21 points versus 0.255 points for patients with baseline MoCA scores ⩾23 (n=141, 80.6%). All other factors had significantly lower significance (Fig. 3) and had no major effect on achieving the primary efficacy endpoint, but the subgroup of patients with greater baseline cognitive impairment tended to have stronger MoCA score changes at the end of the treatment period if administered with SD versus placebo.

Safety analysis was performed in the ITT population. During this study, a total of 307 AEs were recorded after the first dose of the study drug/placebo (Table 4).

All 22 SAEs were unrelated to the study drug/placebo or had only a presumptive or doubtful relation. The outcome of 5 SAEs was fatal (2 cases in the main group and 3 cases in the placebo group). A total of 8 AEs with at least a possible relation to the administered drug were recorded. Among them, there was 1 case of hypersensitivity definitely related to the drug administration. There was also 1 case of leukocytosis, probably related, and 1 case of urinary incontinence, possibly related to the study drug. In the placebo group, there were 5 AEs with possible relation to placebo administration, including leukocytosis, asthenia, dizziness, abdominal pain, and diarrhea (one case of each). All intergroup differences in the incidence of AEs with at least a possible relation to placebo or study drug administration were not significant. Single cases of clinically significant abnormalities in several laboratory parameters, heart rate and overall ECG evaluation were recorded, while no significant intergroup differences in the frequency of clinically significant abnormalities were found either.

Discussion

In a multicenter, double-blind, placebo-controlled randomized trial evaluating the effect of daily use of a succinate-containing neurometabolism-targeting agent on cognitive function, the efficacy of the drug in preventing cognitive impairment after major surgery in elderly patients was shown for the first time to exceed that of placebo. In theory, pharmacological perioperative neuroprotection should reduce the likelihood of neurological, including cognitive, deficits in the postoperative period. In clinics, it has not been demonstrated earlier: the use of lidocaine, ketamine, and magnesium sulfate produced inconsistent results (either questionable effect, or no effect), while there were no differences for the other drugs tested (thiopental, propofol, nimodipine, glutamate/aspartate, xenon, atorvastatin, erythropoietin, piracetam, rivastigmine, estradiol) with respect to POCD between the groups of patients receiving the drug and the control group [22]. Given the ambiguity of POCD diagnostic criteria [23], not the individual neuropsychological tests but an integral MoCA scale, which can assess different cognitive domains (attention and concentration, executive functions, memory, language, visual constructive skills, abstract thinking, counting and orientation) were chosen to evaluate effectiveness, i.e., changes in the total score on this scale occur in any type of cognitive dysfunction. The MoCA scale has high sensitivity and specificity (100% and 87%, respectively) for moderate cognitive dysfunction [13], and has an advantage over other tests in detecting mild cognitive impairment [24]. The results obtained in this study

are consistent with those of previous minor studies that have shown a positive effect of Cytoflavin on cognitive function in patients undergoing cardiac surgery with cardiopulmonary bypass and on the beating heart [10, 11].

To confirm POCD, extensive neuropsychological testing before and after surgery, which should reveal a decrease in cognitive functions in two or more functional domains for at least two weeks, is necessary [15]. However, the extent of this decrease to be considered as clinically significant is controversial, and therefore, there is a strong variation in the frequency of diagnosis of this condition according to different researchers [23]. In this study, we used the proportion of patients with more than a 20% decline in two or more neuropsychological tests at the end of the treatment course as a separate parameter based on the results of a battery of neuropsychological tests including 10 subscales to assess various cognitive domains, which corresponds to the consensus recommendations [15]. The incidence of POCD, according to this definition, on day 31 post-surgery was 11.5% (6.9% of patients in the Cytoflavin® group and 16.2% of patients in the placebo group). This incidence is somewhat lower than in earlier studies [25], which may be due to the smaller portion of patients who underwent cardiac surgery in the study group, exclusion of patients operated with a cardiopulmonary bypass, improvements in general anesthesia techniques and perioperative management. Nevertheless, it is important to note that a significant intergroup difference in favor of the study drug was also observed for this parameter.

Numerous risk factors for postoperative cognitive impairment have been previously described [26], and in addition to older age, initial cognitive deficit, and low educational level, the negative effect of perioperative adverse effects (hypovolemia and cerebral hypoperfusion, arrhythmias, inflammatory reactions, intraoperative blood loss and massive hemotransfusion, reduction of hematocrit after surgery <30%, etc.) were also mentioned [26, 27]. All these factors were considered in this study and studied in a multivariate outcome analysis, which confirmed the relationship between baseline cognitive status and the development of clinically manifest POCD. Other factors had no significant effect on the outcome, probably due to their uncommon occurrence and low severity of adverse intraoperative factors in modern elective surgery.

The results of the study suggest acceptable safety profile of Cytoflavin®: no significant intergroup differences in the frequency of both non-serious and serious AEs were found, and the patterns of AE outcomes were comparable in the study groups. This study limitation is the non-inclusion of cardiac surgery patients operated with extracorporeal circulation, due to lack of an unified protocol of intraoperative extracorporeal circulation in different centers. Although this limitation excluded patients with the highest risk of postoperative cognitive impairment, it helped avoid significant variation in the baseline risk level in the groups, as well as a significant impact of the «center effect» on the final outcome. In addition, patients with postoperative stroke were excluded from the study, since the mechanisms of cognitive dysfunction development in these patients were fundamentally different due to morphological damages to various areas of the brain.

Conclusion

The study drug Cytoflavin® (Inosine + Nicotinamide + Riboflavin + Succinate) demonstrated an acceptable safety profile and helped reduce the severity of cognitive dysfunction in the postoperative period in elderly patients after major surgery, which suggests using the drug for prevention of postoperative cognitive impairment in high-risk patients.

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Metabolomic Profiling of the Blood of Patients with Chronic Disorders of Consciousness

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Summary

The main variants of chronic disorders of consciousness (cDoC) developing in adverse coma outcome are vegetative state/unresponsive wakefulness syndrome (VS/UWS) and minimal consciousness state (MCS).

The aim of the study was to investigate the main differences in metabolomic abnormalities in patients with VS/UWS and MCS, as well as to identify changes in metabolomics depending on sleep or wakefulness phase.

Materials and Methods. Untargeted metabolome analysis of blood plasma of 10 patients in VS/UWS (group 1) and 6 patients in MCS (group 2) was performed using reversed-phase and hydrophilic chromatography methods. The underlying conditions of brain injury were TBI (2 in group 1 and 5 in group 2) and hypoxia (8 in group 1 and 1 in group 2). The internal jugular vein was catheterized in all patients, and blood was collected while awake during the daytime for 2 days. Aliquots of pooled plasma samples were purified from protein components and analyzed by high-performance liquid chromatography in two modes: reversed-phase and hydrophilic ones. Mass-spectrometric detection was performed in full ion current scanning mode: registration of positively charged ions in the m/z range from 50 to 1300 a.u. Data were adjusted and normalized using MS-DIAL software ver. 4.70 software; differences were identified using analysis of variance, discriminant and cluster analysis. The data were analyzed and visualized using MetaboAnalyst 5.0 software (https://www.metaboanalyst.ca).

Results. Four major metabolites (at VIP > 0.5), which content was most modulated depending on the study group, were identified including 4 (*m/z* 124.0867, Rt=17.67, $p<0.01$), 33 (*m/z* 782.5722, *Rt*=17.69, $p<0.01$), 6 (*m/z* 125.0904, *Rt*=18.43, $p<0.01$) and 1 (*m/z* 463.2304, *Rt*=15.78, $p<0.01$), with no significant differences between daytime and nighttime blood samples. Significant quantitative differences were shown for three metabolites in the groups, 14 (*m/z* 162.1126, *Rt*=10.28, $p<0.01$), 35 (*m/z* 780.5483, *Rt*=7.65, $p<0.01$), and 41 (*m/z* 806.5649, *Rt*=7.58, $p<0.01$), and four metabolites when comparing the daytime and nighttime samples: 14 (*m/z* 162.1126, *Rt*=10.28, $p=0.0201$), 35 (*m/z* 780.5483, *Rt*=7.65, $p<0.01$), 41 (*m/z* 806.5649, *Rt*=7.58, $p<0.01$), and 48 (*m/z* 848.5354, *Rt*=7.65, $p<0.01$).

Conclusion. Untargeted metabolomic analysis confirmed the hypothesis of likely significant quantitative and qualitative differences in metabolite composition depending on the type of CCD and circadian rhythm. The study established a set of metabolites that are potential biomarkers for differential diagnosis of VS/UWS and MCS including 4, 33, 6, 1 (in the experiment on the reversed-phase column) and 14, 35, 41, 48 (in the experiment on the hydrophilic column), based on their significant contribution to intergroup and intragroup differences. Further studies will be aimed to characterize the identified metabolites.

Keywords: chronic disorders of consciousness; vegetative state; unresponsive wakefulness syndrome; minimal consciousness state; metabolomics; metabolomic profile; blood-brain barrier; circadian rhythm; glymphatic system; prediction of consciousness recovery; multidisciplinary approach

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Introduction

Chronic disorders of consciousness (cDoC) include variants of adverse coma outcomes, when a patient exhibits no or severe signs of impaired consciousness [1]. The main forms of cDoC include the «vegetative state» or «unresponsive wakefulness syndrome» (VS/UWS), when there is lack of self-awareness and awareness of the environment along with the preserved sleep-wake cycle, as well as the «minimally conscious state minus», when the patient is available for a minimal contact, i. e., visual fixation and eye tracking are present, and a «minimally conscious state plus», when the patient is able to perform a simple task and answer a «yes-no» question verbally or by nodding [2–4].

Significant progress has been made in recent decades in understanding the mechanisms of impaired consciousness after severe brain damage, and a multidisciplinary approach to this problem has been described as a way to bridge the gaps for advancing toward integrated translational science [5]. The most studied and stable functional states of the brain are sleep and wakefulness which occur in circadian rhythms. The study of sleep structure in patients with cDoC becomes particularly relevant due to the discovery of the brain's glymphatic system, which allows the removal of amyloid proteins and tau-oligomers from the glia during the slow sleep phase [6]. Impaired functioning of this system is one of the mechanisms for the development of neurodegenerative processes and neuroinflammation [7–9]. In our opinion, the study of sleep and wake processes in patients with cDoC is particularly interesting from the viewpoint of studying the functional state of the brain, as well as the homeostasis regulation variants supporting these phases. In this respect, metabolomics can be considered as one of the approaches to reveal potential low-molecular-weight biomarkers of the brain performance in patients with cDoC. The general metabolomic profile can serve as a direct indicator of metabolic changes in a biological system [10]. The blood-brain barrier (BBB) is known to be impermeable to most metabolites under normal conditions. Various pathological conditions such as neurodegeneration, neuroinflammation, traumatic brain injury (TBI), and hypoxia result in disruption of the BBB, functioning in both directions; hence, the plasma metabolomic profile may reflect metabolic disorders in the brain [11, 12]. Changes in blood metabolome profile in stroke [13], traumatic brain injury [14], diabetes mellitus [15], and cancer [16–18] have been actively studied.

Analysis of metabolomic research results obtained in the study of sleep and wake phases in patients with cDoC should make it possible to identify additional prognostic markers of potential recovery of consciousness and develop pathogenetic approaches to the treatment of this category of patients in the future. Thus, the aim of this study was to investigate the main differences in metabolomic disorders in patients in the vegetative state/unresponsive wakefulness syndrome and minimally conscious state and identify changes in the metabolome depending on sleep or wake phase.

Material and Methods

The study was supported by the ethical committee of the V. A. Almazov Scientific Research Center (protocol No. 23082019) and was conducted in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki). Patients stayed in the anesthesiology and intensive care unit for at least 21 days (in 2019–2020). Sixteen patients with cDoC were included in the study. To evaluate the signs of consciousness, the patients were assessed on the Coma Recovery Scale- Revised (CRS-R) 5 times during the first 10 days of hospitalization. Depending on the total CRS-R score, patients were divided into two groups. Group 1 included patients with a CRS-R score of 0 to 5 (their level of consciousness met the VS/UWS criteria), and Group 2 included patients with a CRS-R score of 9 to 15 (their level of consciousness met the MCS «minus» or «plus» criteria). Characteristics of patients by age, duration of consciousness disorder, and total CRS-R score are shown in Table 1.

The causes of brain damage included TBI (2 in group 1 and 5 in group 2) and hypoxia (8 in group 1 and 1 in group 2). Patients with acute infection, hepatic and renal dysfunction were not included in the study. Medications received by the patients at the time of serum sampling are presented in Table 2.

The study steps are shown in Fig. 1.

A catheter was inserted into the right jugular vein in all patients a day before the examination, with the catheter tip directed upward (against the blood flow) — to obtain blood «flowing out» from the brain. Blood sampling was performed in the awake state at daytime (at 12:00) and at night (at 3:00 am) for 2 days (two daytime and two nighttime samplings were performed for 2 days). Blood was collected in vacutainers with EDTA as an anticoagulant, placed on ice, and immediately taken to the laboratory, where it was centrifuged for 15 minutes at 2700 rpm, aliquoted in Eppendorf tubes, and frozen at –25°C. Hemolyzed samples were discarded and not included in further study.

Preparation of samples. Pooling and purification of blood plasma from protein molecules. Pooled blood plasma samples were used for analysis. Pools were formed at the daytime and nighttime points for each group, i.e., 2 pooled

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Table 1. Characteristics of patients with chronic disorders of consciousness.

Main parameters	Age, years	Duration of disorder of consciousness, months	CRS, points
Group 1 (*n*=10)			
Mean	38	5.4	4
Standard deviation	12	7.8	1
Median	40	2.0	4
Minimum	21	1.0	1
Maximum	54	26.0	5
25th percentile	26	1.0	3
75th percentile	45	6.0	5
Group 2 (*n*=6)			
Mean	37	9.7	12
Standard deviation	15	11.3	2
Median	31	4.0	11
Minimum	21	1.0	9
Maximum	61	26.0	15
25th percentile	29	1.0	10
75th percentile	51	22.0	13

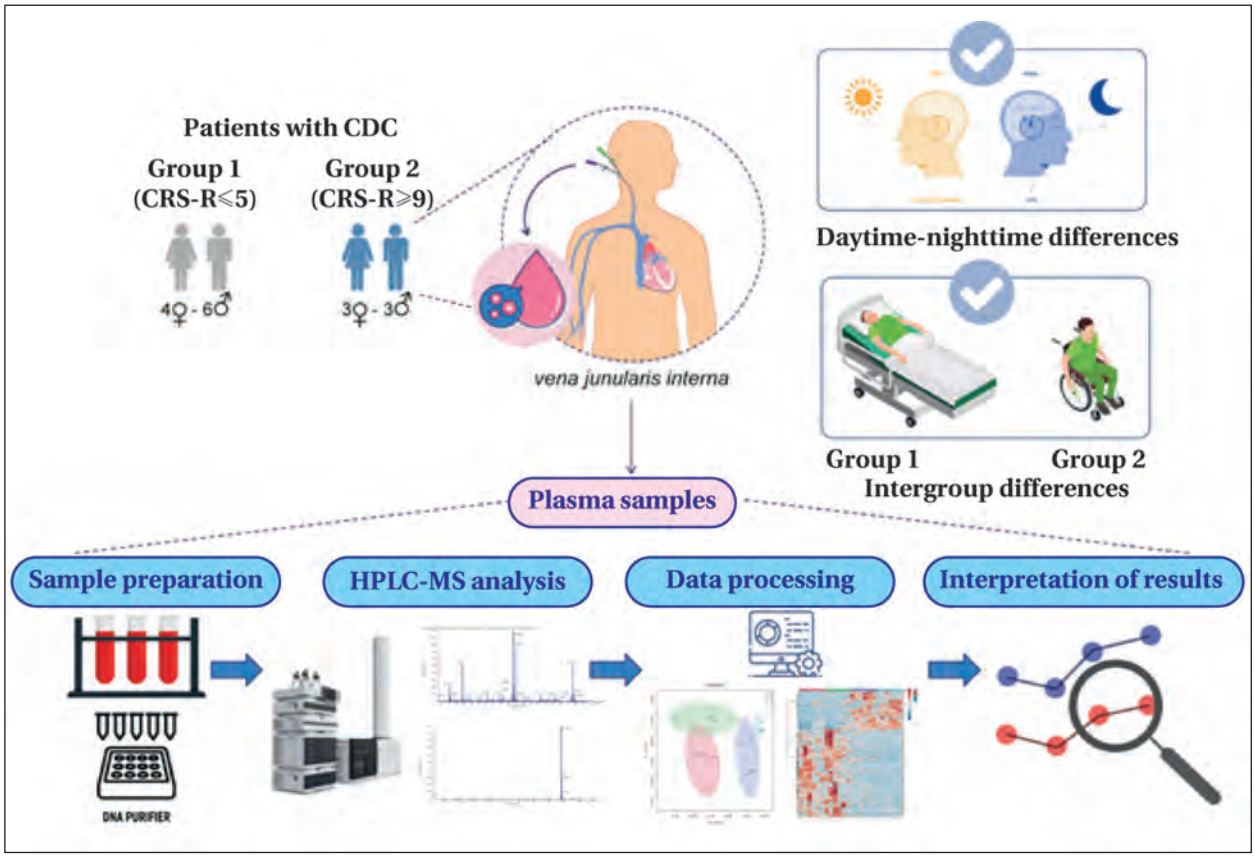

Fig. 1. The steps of the non-targeted blood test in patients with chronic disorders of consciousness.

samples were formed in Group 1 (daytime sample contained the plasma of 10 patients of first and second day sampling; nighttime sample contained the plasma of 10 patients of first and second nighttime sampling) and 2 pooled samples in Group 2 (daytime sample contained the plasma of 6 patients of first and second daytime sampling; nighttime sample contained the plasma of 6 patients of first and second nighttime sampling). An aliquot of 100 μL was taken from each pooled sample and transferred to a new Eppendorf tube. Then, 400 μl of chilled acetonitrile was added to each sample and stirred. To remove protein components, samples were centrifuged for 10 min (12,000 rpm, 4°C), and 400 μl of supernatant was transferred to a chromatographic vial for subsequent analysis.

Chromatography and mass spectrometry. Conditions of chromatographic separation. The chromatographic separation of the components

Table 2. List of medications received by patients from the study groups.

Group 1 (VS/UWS)	Group 2 (MCS)
Ipidacrine	
Amlodipine	
Bisoprolol	
Valproic acid	
Phenytoin	
Ethylmethylhydroxypyridine succinate	
Amantadine sulfate	
Nadroparin calcium	
Tolperisone hydrochloride	
Choline alfoscerate	
Citicoline	
Omeprazole	
Spironolactone	Lactulose
Levetiracetam	Clonidine
Memantine	Proroxan
Pancreatin	Brain peptide complex
Carbamazepine	
Fluconasole	
Succinic acid, inosine, nicotinamide	
Apixaban	
Levothyroxine	

Table 3. Ratio of mobile phase components.

Time, minutes	A,%	B,%
0	95	5
0.5	95	5
15.5	45	55
16.5	25	75
17.5	25	75
17.6	95	5
20	95	5

Note. Eluent flow rate = 0.3 ml/min; column thermostat temperature = 40°C; thermostat temperature (sampling department) = 10°C; sample injection volume — 2 µl; analysis time — 20 min.

Table 4. Ratio of mobile phase components.

Time, minutes	A,%	B,%
0	5	95
3	5	95
12	40	60
15	40	60
16	5	95
20	5	95

Note. Eluent flow rate = 0.3 ml/min; column thermostat temperature = 40°C; thermostat temperature (sampling department) = 10°C; sample injection volume — 2 µl; analysis time — 20 min.

using high-performance liquid chromatography (HPLC) was performed in two modes:

1. Reverse phase chromatography

- Intensity Solo 2 C18 (Bruker) column, length 100 mm, column diameter 2.10 mm, sorbent particle diameter 1.8 µm.
- The mobile phase:
  - component A — 0.1% solution of formic acid in deionized water;
  - component B — HPLC category acetonitrile;
- Gradient chromatographic elution mode

Hydrophilic interaction liquid chromatography (HILIC)

- Ascentis Express HILIC (Merck) column, length 100 mm, column diameter 2.10 mm, diameter of sorbent particles 2.7 µm
- Mobile phase:
  - component A — 10 mM NH4COOH with added formic acid (0.1%) in deionized water;
  - component B — HPLC category acetonitrile;
- Gradient chromatographic elution mode

Operating conditions of the mass spectrometric detector. Mass spectrometric detector was Bruker Q-TOF Maxis Impact with electrospray ionization at atmospheric pressure and «otof Control» software for data control and processing.

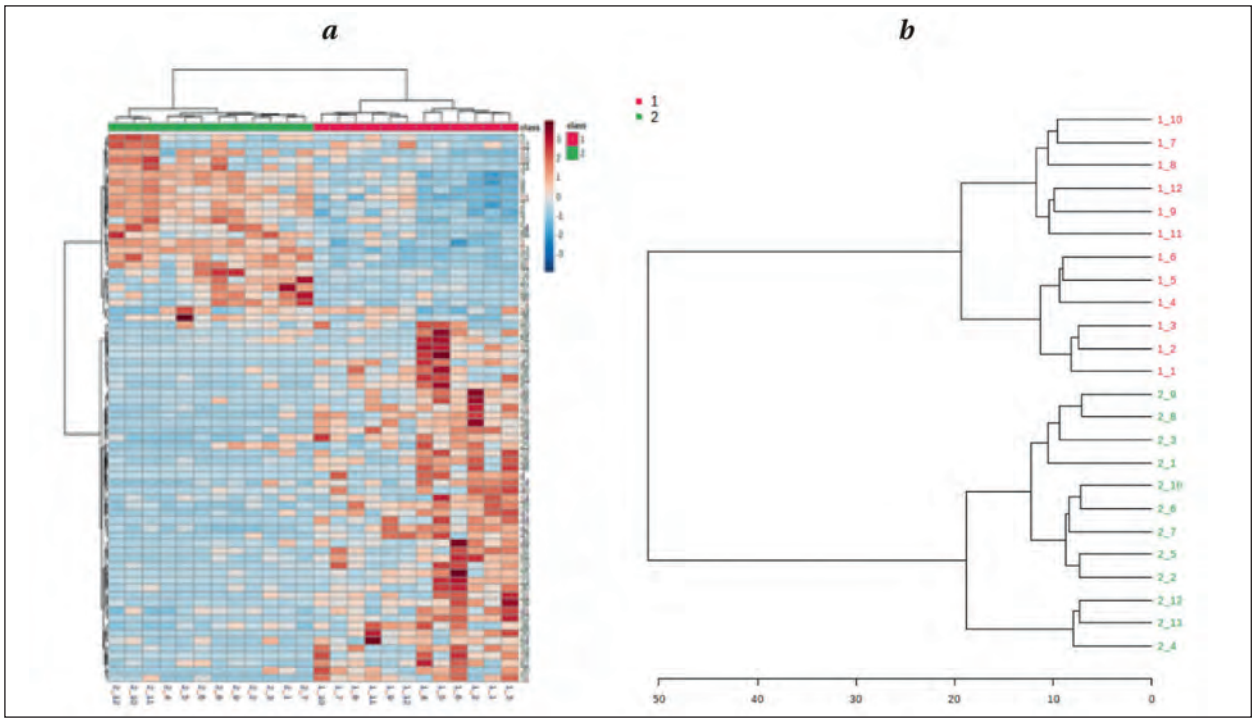

Fig. 2. Hierarchical cluster analysis of samples from groups 1 and 2 from the C18 column.
Note. *a* — heat map of metabolites. The studied plasma samples by groups are arranged in columns: red correspond to group 1, green to group 2, and the identified component variables are arranged in rows. The colors range from blue to red, indicating a change in the content of the variables from very low (dark blue) to high (dark red). *b* — dendrogram. Samples form clusters of groups 1 (red) and 2 (green).

Mode: drying gas flow (nitrogen) 8 l/min; gas pressure on the nebulizer 2 bar; conducting capillary temperature 220°C; capillary voltage 4500 V; detection in full ionic current scanning mode: registration of ions in the m/z range from 50 to 1300 a.u. (with positive ionization).

Data processing. Data from the chromatomass spectrometric analysis were converted to mzML format and processed using MS-DIAL metabolomics software (http://prime.psc.riken.jp/Metabolomics_Software/MS-DIAL/index.html). Data adjustment, normalization, and filtering (the data were cleared of background noise and unrelated ions) were performed using MS-DIAL software ver. 4.70. The concentrations of the studied substances by definition have a log-normal distribution, so the data were subjected to median normalization, logarithmic transformation, and automatic scaling (centeraveraged and divided by the standard deviation of each variable). Data were statistically processed and visualized using the MetaboAnalyst 5.0 platform (https://www.metaboanalyst.ca/), which is generally accepted for metabolic analysis. The intergroup differences were tested using Student's test, Mann-Whitney test (to compare groups 1 and 2), and ANOVA test (to compare circadian changes of metabolomics) in accordance with normally distributed logarithmically transformed variables. The critical level of significance was set at α=0.05. In addition, we performed unsupervised principal component analysis (PCA) and supervised PLS-DA analysis with a Pareto-scaled data set and power transformation using the first two latent variables. Based on the PLS-DA models, we created volcano plots showing the importance of variables in projection (VIP) versus adjusted p-values [p (corr), load values scaled as correlation coefficient values]. Variables with VIP > 0.5, q ⩽ 0.050, and absolute p (corr) ⩾ 0.30 were considered significant. To illustrate complex associations between several parameters based on normalized data, cluster analysis was performed, in which clustering was performed using the Ward method, and the Euclidean distance was considered as a measure of proximity [19, 20].

Results

Initial data processing. Pooled blood samples from cDoC patients of two groups were analyzed. High-performance liquid chromatography with mass spectrometry detector (HPLC-MS) data for all pooled samples were obtained on two different chromatographic columns, hydrophilic (HILIC) and reversed-phase (C18), for comprehensive evaluation of metabolites of different polarity. Group spectra

showed a consistent set of metabolite signals present. The number of raw spectral signals detected instrumentally was 6569 for HILIC analysis and 7952 on the C18 column. Noise and artifact peaks were filtered out, yielding 6134 and 4759 signals, respectively. Based on the hypothesis of probable significant quantitative and qualitative differences in the composition of metabolites depending on the severity of patient condition, we identified those metabolites whose relative levels, calculated from chromatographic peak areas, dempnstrated significant intergroup differences, using ANOVA analysis. Thus, we identified 63 metabolites for the HILIC column and 73 metabolites for the C18 column (P<0.05) (Table 5, 6 — see Appendix).

Analysis of reversed-phase chromatography data. Normalized data of the relative intensities of the chromatographic signals obtained on the C18 column were imported into MetaboAnalyst 5.0 software and visualized using cluster analysis techniques. The resulting hierarchically grouped heat map of metabolites reflects a comparative assessment of mean metabolite contents, and the dendrogram demonstrates, on the one hand, a significant correlation between all samples from one group, and a distinct clustering of samples belonging to groups 1 and 2 on the other hand (Fig. 2).

Preliminary conclusions were confirmed using other statistical methods. An estimation plot based on the unsupervised PCA model (Fig. 3, *a*) showed clusters of Group 1 (VS/UWS) and Group 2 (MCS) samples in the two main components PC1 and PC2, which accounted for 87% and 12% of the variance in the data, respectively. To make this difference more apparent, we performed a supervised PLS-DA analysis with a Pareto-scaled data set and power transformation using the first two latent variables. This model produced clusters similar to the PCA for groups 1 and 2 (Fig. 3, *b*), and both models showed relatively greater homogeneity of group 2 samples. According to the PLS-DA loading plots (Fig. 3, *c*), we identified four major metabolites (at VIP > 0.5) whose content was most modulated depending on the patient group, which included signal 4 (m/z 124.0867, Rt=17.67, P<0.01), 33 (m/z 782.5722, Rt=17.69, P<0.01), 6 (m/z 125.0904, Rt=18.43, P<0.01), and 1 (m/z 463.2304, Rt=15.78, P<0.01). Metabolite 4 content was higher in group 1, while the content of metabolites 33, 6, and 1 was higher in group 2.

When analyzing differences in the composition of metabolites of daytime and nighttime samples on column C18, no significant differences were found. Hierarchical cluster analysis showed a significant correlation between both daytime and nighttime samples of the same group (Fig. 4, *a* and *b*), no isolated clusters of daytime and nighttime sampling appeared in the PLS-DA analysis (Fig. 5).

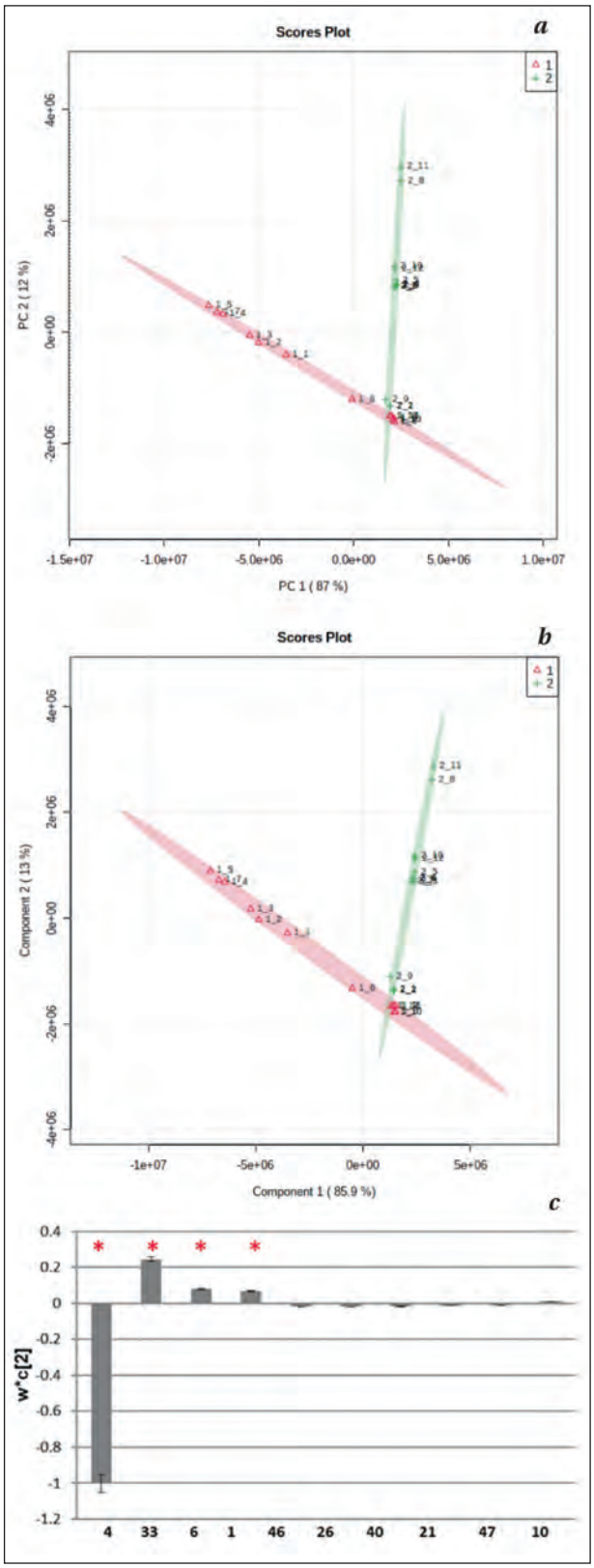

Fig. 3. Graphs of the assessment of PCA (A) and PLS-DA (B) in groups 1 and 2 from C18 column.
Note. In Figures *a* and *b*, the red cloud represents the cluster of group 1, the green one — the cluster of group 2. 1_1-1_12 — studied plasma samples from patients of group 1; 2_1-2_12 — studied plasma samples from patients of group 2. *b* — PLS-DA model load graph observed by PC 2 (w * c [2]): each column represents a putative marker metabolite with standard errors displayed in the error bar.

Table 5. List of target metabolites for the HILIC column

Metabolite No.	m/z	tR	Normalized mean for group 1	Normalized mean for group 2	*P* value
1	116.07095	2.488	0.723±0.626	–0.723±0.747	<0.0001*
2	121.96481	8.845	0.730±0.221	–0.730±0.938	0.0002*
3	129.06573	9.348	0.800±0.507	–0.800±0.662	<0.0001*
4	129.97771	10.045	–0.549±1.025	0.549±0.617	0.0018**
5	131.95901	8.258	–0.209±0.593	0.209±1.282	0.0284**
6	133.95724	8.564	–0.864±0.569	0.864±0.374	<0.0001*
7	136.04131	3.363	0.038±1.257	–0.038±0.712	0.3777**
8	143.99974	10.047	–0.607±1.100	0.607±0.275	0.0028*
9	144.98315	6.978	–0.766±0.891	0.766±0.129	<0.0001*
10	146.1156	10.44	–0.275±0.971	0.275±0.991	0.1842*
11	146.11641	10.411	–0.265±0.972	0.265±0.996	0.2005*
12	153.03342	9.78	–0.766±0.859	0.766±0.271	<0.0001**
13	156.04485	9.013	–0.908±0.463	0.908±0.278	<0.0001*
14	162.11261	10.278	0.206 ±0.778	–0.206±1.180	0.3228*
15	175.11806	10.621	–0.823±0.557	0.823±0.552	<0.0001*
16	185.02979	1.43	–0.012±0.954	0.012±1.086	0.955*
17	188.07005	7.322	–0.408±0.695	0.408±1.116	0.0427*
18	226.04523	7.582	–0.478±0.821	0.478±0.958	0.0155*
19	353.07663	3.476	–0.695±0.381	0.695±0.944	0.0003*
20	385.03558	7.325	0.075±0.898	–0.0750±0.128	0.2657**
21	480.34351	8.566	–0.853±0.482	0.853±0.520	<0.0001*
22	496.62271	8.726	0.369±0.221	–0.369±1.321	0.0284**
23	533.32251	3.401	0.369±0.623	–0.369±1.186	0.0145**
24	566.89081	7.513	–0.860±0.584	0.860±0.370	<0.0001*
25	634.87213	7.513	–0.899±0.398	0.899±0.413	<0.0001*
26	701.49713	7.245	–0.898±0.468	0.898±0.333	<0.0001*
27	702.85931	7.513	–0.884±0.428	0.884±0.452	<0.0001*
28	736.86053	7.512	–0.879±0.431	0.879±0.469	<0.0001*
29	738.50195	6.442	0.473±0.087	–0.473±1.263	0.0029**
30	741.53412	8.296	–0.884±0.471	0.884±0.407	<0.0001*
31	759.04663	7.651	0.663±0.486	–0.663±0.946	0.0005*
32	759.17041	7.65	0.523±0.794	–0.523±0.929	0.0071*
33	769.55829	7.652	–0.773±0.694	0.773±0.553	<0.0001*
34	770.85394	7.513	–0.884±0.444	0.884±0.434	<0.0001*
35	780.54828	7.654	–0.873±0.515	0.873±0.403	<0.0001**
36	784.93988	7.592	0.642±0.373	–0.642±1.026	0.0012*
37	786.96295	7.596	0.236±1.240	–0.236±0.657	0.2593*
38	793.54767	8.297	–0.901±0.457	0.901±0.333	<0.0001*
39	796.54877	7.777	–0.812±0.611	0.812±0.527	<0.0001*
40	802.58978	1.545	–0.017±1.071	0.017± 0.971	0.936*
41	806.56488	7.584	–0.823±0.593	0.823±0.510	<0.0001*
42	814.55292	7.813	–0.741±0.906	0.741±0.268	<0.0001**
43	815.54834	7.522	0.556±0.475	–0.556±1.091	0.0055*
44	818.52917	7.645	–0.655±1.040	0.655±0.271	<0.0001**
45	818.59137	7.579	0.101±0.874	–0.101±1.143	0.9774**
46	833.64746	8.141	0.255±1.098	–0.255±0.862	0.1005**
47	843.57544	7.474	–0.878±0.505	0.878±0.392	<0.0001*
48	848.5354	7.654	0.831±0.473	–0.831±0.602	<0.0001*
49	852.55658	5.647	0.456±0.413	–0.456±1.211	0.0100**
50	854.55298	7.578	0.257±0.569	–0.257±1.274	0.5899**
51	856.5672	7.58	–0.299±1.197	0.299±0.681	0.1473*
52	862.54602	7.497	–0.949±0.272	0.949±0.223	<0.0001*
53	867.07965	7.481	0.286±0.545	–0.286±1.271	0.5137**
54	868.52759	7.404	–0.827±0.555	0.827±0.537	<0.0001*
55	874.5517	7.581	0.644±0.383	–0.644±1.020	0.0011*
56	876.55682	5.555	–0.892±0.465	0.892±0.370	<0.0001**
57	876.57294	7.59	0.552±0.457	–0.552±1.103	0.0002**
58	878.56982	5.604	–0.112±1.418	0.112±0.228	0.1432**
59	880.58649	5.616	0.121±0.765	–0.121±1.214	0.8874**
60	896.56024	7.365	–0.868±0.454	0.868±0.489	<0.0001*
61	906.8288	7.513	–0.203±0.888	0.203±1.101	0.3307*
62	922.5542	7.445	–0.879±0.433	0.879±0.468	<0.0001*
63	974.80841	7.513	0.723±0.626	–0.723±0.747	<0.0001*

Note. Here and in Table 6: * — *P* value calculated using Student's *t*-test; ** — *P* value calculated using the Mann–Whitney test.

Table 6. List of target metabolites for the C18 column.

Metabolite No.	m/z	tR	Normalized mean for group 1	Normalized mean for group 2	*P* value
1	15.78	463.2304	–0.755±0.768	0.755±0.506	<0.0001**
2	18.65	108.0811	–0.798±0.485	0.798±0.684	<0.0001*
3	18.77	118.0872	–0.708±0.912	0.708±0.406	<0.0001**
4	17.67	124.0867	–0.257±1.062	0.257±0.905	0.3777*
5	17.93	124.1716	–0.692±0.639	0.692±0.799	0.0001*
6	18.43	125.0904	–0.803±0.665	0.803±0.493	<0.0001*
7	18.78	128.9628	–0.753±0.757	0.753±0.529	<0.0001*
8	19.05	131.9308	–0.711±0.884	0.711±0.453	<0.0001**
9	18.71	131.9625	–0.812±0.518	0.812±0.620	<0.0001*
10	17.75	138.102	–0.671±0.725	0.671±0.764	0.0002*
11	18.71	147.0929	–0.731±0.888	0.731±0.369	0.0001*
12	18.82	182.9617	–0.357±0.820	0.357±1.068	0.1005**
13	18.77	90.5081	–0.883±0.422	0.883 ±0.460	<0.0001*
14	18.7	674.4291	–0.004±1.151	0.004±0.876	0.7728**
15	18.72	686.6918	0.404±1.271	–0.404±0.344	0.5443**
16	18.68	711.7711	0.675 ±0.727	–0.675±0.753	0.0007**
17	18.72	716.5522	–0.705±0.826	0.705±0.570	<0.0001*
18	17.75	723.4638	0.341±1.234	–0.341±0.561	0.4356**
19	18.54	727.4619	–0.622±0.764	0.622±0.814	0.0008*
20	18.66	727.7125	0.159 ±1.178	–0.159±0.806	0.3863**
21	18.66	738.4745	0.488 ±0.581	–0.488±1.111	0.0156*
22	18.72	741.2301	0.636±0.899	–0.636±0.632	0.0262**
23	18.65	748.7361	0.290±1.240	–0.290 ±0.608	0.3122**
24	17.73	755.4784	0.519±0.581	–0.519±1.080	0.0376**
25	17.78	757.9766	–0.250±1.040	0.250±0.935	0.2288*
26	17.72	763.49	0.286±1.041	–0.286± 0.911	0.1409**
27	18.63	772.2356	0.530±0.749	–0.530±0.957	0.0193**
28	17.56	772.4897	0.551±1.029	–0.551±0.608	0.0056**
29	18.71	772.493	–0.010 ±1.187	0.010±0.825	0.5066**
30	18.7	773.4938	0.043±1.031	–0.043±1.012	0.8852**
31	18.71	778.0045	0.345 ±1.088	–0.345±0.804	0.0734**
32	17.93	778.9939	0.210±1.166	–0.210±0.797	0.4095**
33	17.69	782.5722	–0.790±0.725	0.790±0.452	<0.0001**
34	18.7	782.7464	0.418±0.530	–0.418±1.195	0.1600**
35	17.9	792.5078	0.375±0.663	–0.375±1.160	0.0646*
36	18.71	793.3143	0.068±1.002	–0.068±1.037	0.7475*
37	18.67	800.6866	–0.755±0.662	0.755±0.639	<0.0001*
38	17.91	802.0062	–0.777±0.573	0.777±0.667	<0.0001*
39	17.66	805.7479	0.457±0.630	–0.457±1.113	0.0209**
40	17.8	808.5083	0.026±1.215	–0.026±0.784	0.8874**
41	18.64	813.5074	–0.108±0.921	0.108±1.103	0.6085*
42	17.95	816.5212	0.325±0.392	–0.325±1.306	0.1224*
43	17.65	821.1963	0.532±0.836	–0.532±0.880	0.0145**
44	17.93	821.8597	0.692±0.936	–0.692±0.410	0.0003*
45	17.7	829.8372	0.669±0.958	–0.669±0.442	0.0043**
46	17.79	831.8625	0.566±1.008	–0.566±0.613	0.0031*
47	18.67	831.863	0.470±0.869	–0.470±0.923	0.0145**
48	17.76	848.8497	0.589±1.037	–0.589±0.507	0.0028*
49	17.89	849.0341	0.293±0.961	–0.293±0.990	0.1550*
50	18.7	855.7933	0.257±1.180	–0.257±0.744	0.2151*
51	18.71	857.2792	0.379±0.753	–0.379±1.100	0.2144**
52	18.62	862.7601	–0.858±0.582	0.858±0.384	<0.0001*
53	18.58	863.1935	0.401±1.020	–0.401±0.837	0.1938**
54	18.66	867.7538	–0.747±0.616	0.747±0.703	0.0002**
55	18.63	877.3093	0.546±0.906	–0.546±0.787	0.0086**
56	17.87	886.5561	0.374±1.072	–0.374±0.798	0.1135**
57	18.63	887.5426	–0.826±0.445	0.826±0.637	<0.0001**
58	18.64	896.2261	–0.008±1.236	0.008±0.751	0.7125**
59	17.83	897.5506	–0.119±0.990	0.119±1.039	0.5726*
60	18.65	915.5733	–0.012±1.010	0.012±1.034	0.6297**
61	18.71	919.8516	–0.814±0.762	0.814±0.259	<0.0001*
62	18.57	926.2381	0.168 ±0.949	–0.168±1.062	0.6033**
63	18.69	928.9295	0.581±1.026	–0.581±0.548	0.0086**
64	17.96	935.5851	0.221±0.951	–0.221±1.039	0.5137**
65	17.64	935.9096	0.547±0.637	–0.547±1.016	0.0121**
66	18.64	948.9295	–0.853±0.402	0.853±0.583	<0.0001**
67	17.99	950.2549	0.634±0.228	–0.634±1.078	0.0018*
68	18.71	977.2341	–0.792 ±0.719	0.792±0.453	<0.0001*
69	18.65	982.1268	0.572±0.711	–0.572±0.934	0.0027*
70	18.63	1007.1024	0.398±1.014	–0.398 ±0.846	0.0605**
71	17.79	1033.6344	–0.835±0.665	0.835±0.356	<0.0001*
72	18.57	1036.2665	–0.736±0.804	0.736±0.513	<0.0001**
73	17.79	1056.1636	0.249±1.217	–0.249±0.690	0.2306*

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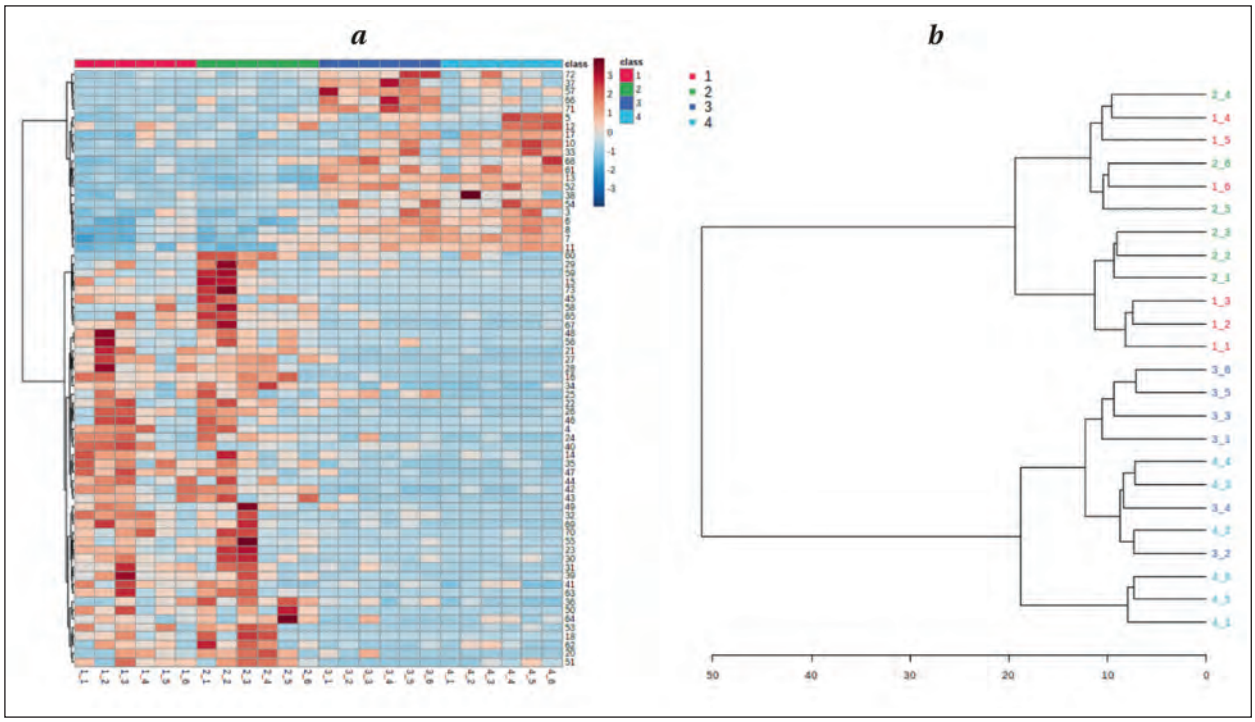

Fig. 4. Hierarchical cluster analysis of samples of daytime and nighttime sampling in the studied groups from C18 column.
Note. *a* — heat map of metabolites. The studied plasma samples are arranged by groups in the columns: red correspond to daytime sampling from group 1, green — nighttime sampling from group 1; dark blue — daytime sampling from group 2, blue — nighttime sampling from group 2, and the identified component variables are arranged in rows. The colors range from blue to red, indicating a change in the content of the variables: from very low (dark blue) to high (dark red). *b* — dendrogram: 1 — group 1, daytime sampling; 2 — group 1, nighttime sampling; 3 — group 2, daytime sampling; 4 — group 2, nighttime sampling. The samples form clusters of groups 1 and 2, but do not show a distinct clustering into daytime and nighttime sampling.

Analysis of hydrophilic chromatography data. The hierarchically grouped heat map of the metabolites with the highest relative content of the chromatographic signals obtained on the HILIC column and the dendrogram shows results similar to those obtained on the C18 column. All samples of the same group showed a significant correlation between each other when separating groups 1 and 2 into separate clusters (Fig. 6, *a, b*). However, when analyzing the data from the samples taken at different times, we observed them forming separate clusters indicating the presence of significant differences in the metabolic profiles of daytime and nighttime samples (Fig. 6, *c, d*).

The PCA and PLS-DA assessment plots showed distinct clustering of groups 1 and 2 in the two major components PC1 and PC2, which accounted for 99% and 0.5% of sample variance in PCA analysis (Fig. 7, *a*) and 99% and 0.3% in PLS-DA analysis (Fig. 7, *b*). The PLS-DA loading plots (Fig. 7, B) identified three major metabolites (at VIP > 0.5) most significant for clustering groups in the PLS-DA model, namely 14 (m/z 162. 1126, *Rt*=10.28, p < 0.01), 35 (m/z 780.5483, *Rt*=7.65, *P*<0.01), and 41 (m/z 806.5649, *Rt*=7.58, *P*<0.01), whose content was higher in group 1. When comparing daytime and nighttime sampling, however, there was marked

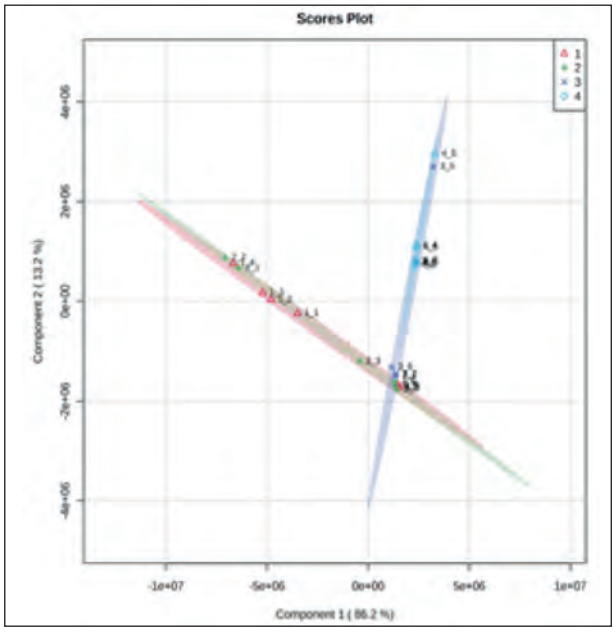

Fig. 5. PLS-DA analysis of differences between samples 1 and 2 with the daytime and nighttime sampling from C18 column.
Note. Red cloud represents group 1, daytime sampling; green cloud is group 1, nighttime sampling; dark blue cloud is group 2, daytime sampling; blue cloud is group 2, nighttime sampling. Red and green, dark blue and blue clouds overlap in pairs, which indicates the presence of intergroup and the absence of intragroup differences depending on the time of collection.

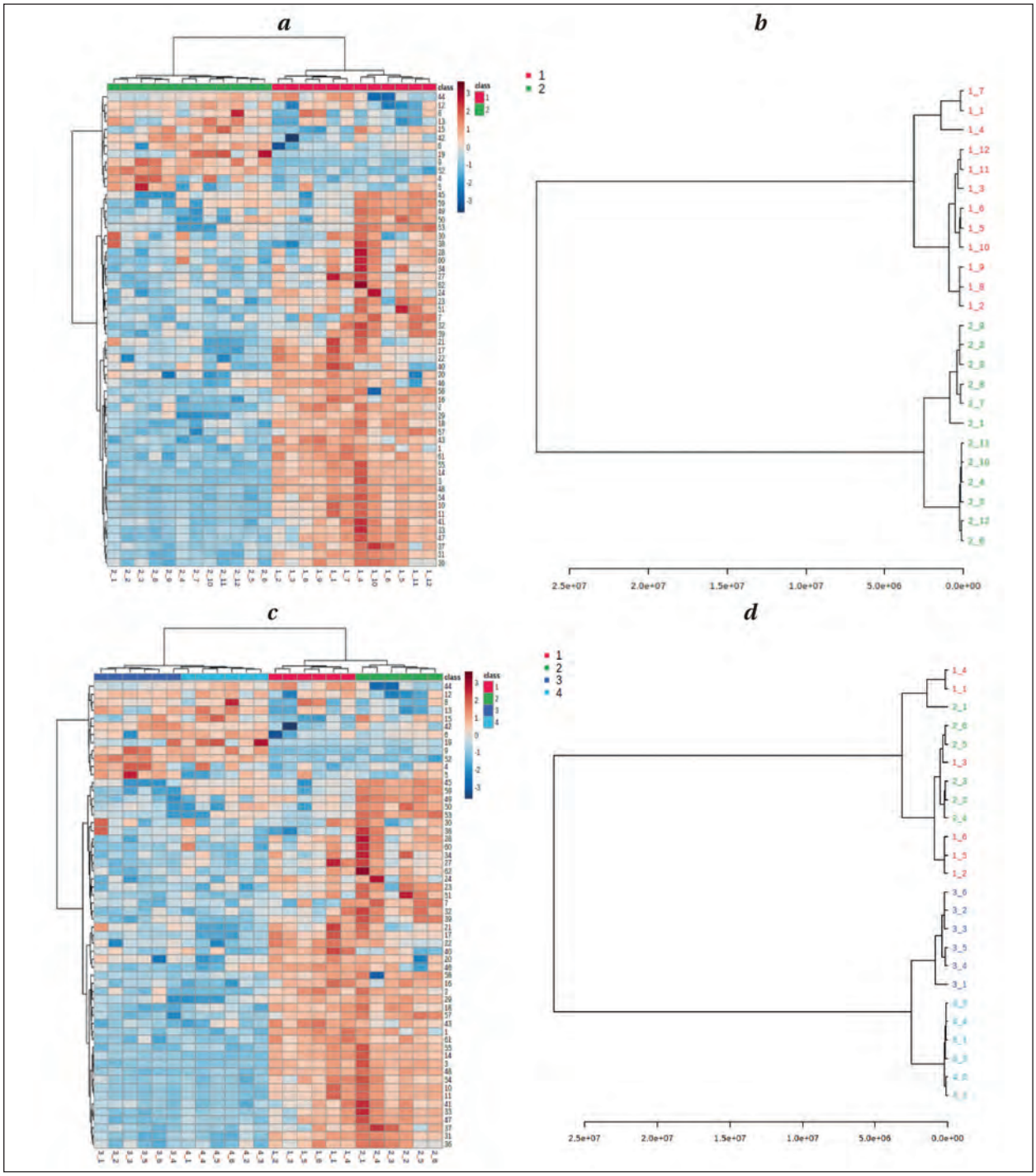

Fig. 6. Hierarchical cluster analysis of samples from the HILIC column.
Note. *a* — heat map of the studied metabolites of groups 1 and 2. The studied plasma samples by groups are arranged in the columns. Red color indicated group 1, green — group 2, and the identified component variables are arranged in rows. The colors range from blue to red, indicating a change in the content of the variables from very low (dark blue) to high (dark red). *b* — Dendrogram showing samples forming distinct clusters of groups 1 (red) and 2 (green). *c* — heat map of metabolites for comparison of daytime and nighttime sampling of the group: red corresponds to group 1 with the daytime sampling, green — to the group 1 with the nighttime sampling; dark blue represents group 2 with the daytime sampling, blue color shows group 2 with nighttime sampling, and the identified component variables are arranged in rows. The colors range from dark blue to dark red, indicating a change in the content of the variables from very low (dark blue) to high (dark red). *d* — dendrogram: 1 — group 1, daytime sampling; 2 — group 1, nighttime sampling; 3 — group 2, daytime sampling; 4 — group 2, nighttime sampling. Samples form clusters of groups 1 and 2, daytime and nighttime sampling.

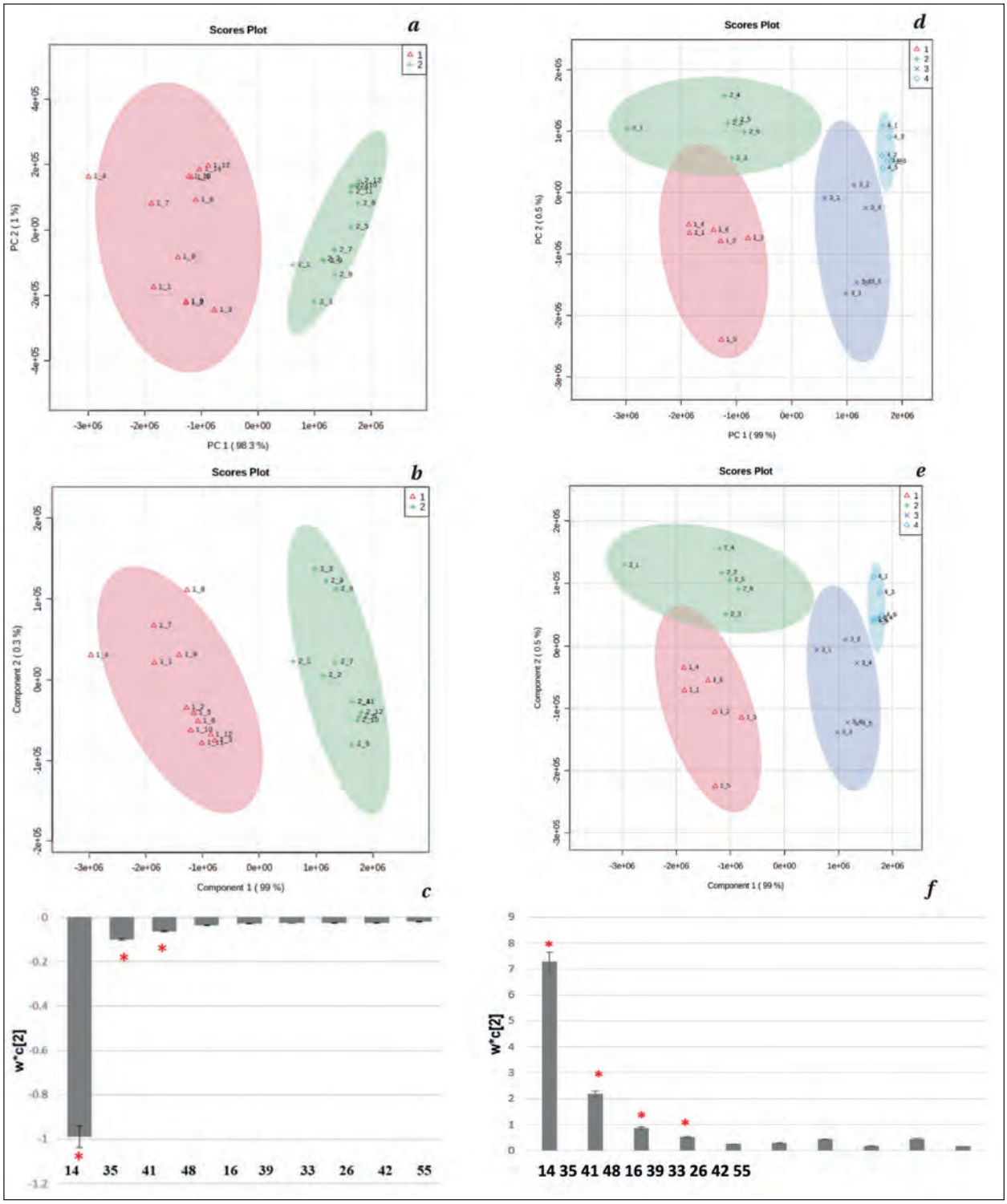

Fig. 7. Graphs of PCA (A) and PLS-DA (B) of groups 1 and 2 (HILIC).
Note. Group 1 cluster is represented by red cloud, Group 2 cluster is represented by green cloud. *a* — studied plasma samples of patients in group 1; *b* — studied plasma samples of patients in group 2. *c* — PLS-DA model loading plot, PC 2 (w*c [2]): each column represents the putative marker metabolite with standard errors displayed on the error panel. PCA (*d*) and PLS-DA (*e*) plots of differences between samples of daytime and nighttime sampling in groups 1 and 2 from column C18: red cloud, daytime sampling from group 1; green cloud, nighttime sampling from group 1; blue cloud, daytime sampling from group 2; blue cloud, nighttime sampling from group 2. Four separate clouds are formed, indicating significant differences both between groups and between samples at different times of the day. *f* — is the PLS-DA model loading plot, PC 2 (w*c [2]): each column represents the putative marker metabolite with standard errors displayed in the error panel.

clustering of samples both between and within the groups, depending on the time of sampling, with a slight overlap in the second component (Fig. 7, *d*). The PLS-DA model produced results similar to those of the PCA model (Fig. 7, *e*). Based on PLS-DA load plots (Fig. 7, *f*), we identified metabolites (at VIP > 0.5) that played a key role in intragroup differences, including 14 (m/z 162.1126, *Rt*=10.28, *P*=0.0201), 35 (m/z 780.5483, *Rt*=7.65, *P*<0.01), 41 (m/z 806.5649, *Rt*=7.58, *P*<0.01) and 48 (m/z 848.5354, *Rt*=7.65, *P*<0.01), whose levels were higher in Group 2 patient samples.

Discussion

As a result of non-targeted metabolome analysis of pooled plasma samples of patients in VS/UWS (group 1) and in MCS (group 2), we revealed several unidentified compounds, whose levels are most strongly associated with the type of cDoC (VS/UWS or MCS). To the best of our knowledge, this study was the first to compare «daytime» and «nighttime» plasma metabolome of patients with cDoC. Our findings have indicated significant differences between the studied samples, which suggests the possibility of identifying prognostic and diagnostic markers in the future research. However, it also became evident that non-targeted metabolomic analysis is not informative enough.

The first study on metabolomics in patients with cDoC was conducted by Jie Yu et al. in 2021 [21]. The authors used nontargeted and targeted plasma metabolome analysis in patients in VS/UWS and MCS to identify the main metabolomic abnormalities in patients of these two groups. Their findings showed that the metabolomic profile of patients with cDoC differed significantly not only from that of healthy volunteers, but also between patients in VS/UWS and MCS, with particularly relevant differences found in the lipidome analysis. The authors identified certain lipids whose levels were significantly elevated in patients in VS/UWS and MCS. For example, there was a significant difference in phosphatidylcholine and arachidonic acid levels between patients in VS/UWS and MCS, which, according to the authors, could serve as a marker for differential diagnosis of these disorders of consciousness. Also, significant differences in purine metabolism were observed in patients with cDoC compared to the control group of healthy volunteers. Patients in VS/UWS and MCS demonstrated decreased levels of adenosine, adenosine diphosphate, and adenosine monophosphate, which resulted from the adenosine triphosphate degradation. The results of other studies of metabolomics in patients with acute and subacute TBI have also shown that lipidomics was the most promising study [22, 23].

In a recently published paper by T. Dawiskiba et al addressing the metabolomic profile of patients diagnosed with brain death or coma, proline, orthophosphoric acid, β-hydroxybutyric acid, galactose, creatinine, valine, linoleic acid, arachidonic acid, medium-chain fatty acids were found to be both markers of acute traumatic brain damage and adverse outcome (death) predictors [24, 25]. Studies There are few studies on metabolomics in patients post hypoxic brain injury [26, 27]. Apparently, potential metabolomic markers in hypoxic damage would be those identified in ischemic stroke sych as lactate, pyruvate, glycolic acid, formate, glutamine, methanol, acetate, cysteine, folic acid, tyrosine, tryptophan, valine, carnitine, etc. [25, 28–30].

Thus, metabolomics is an rapidly evolving area of modern translational medicine [31–35]. Metabolomic changes can be minor and consist in the abnormal ratios between various chemical compounds, however, it is possible to identify some completely new chemical compounds («disease biomarkers») and/or loss of certain molecules normally present in the homeostatic state [36-38]. We believe that the study of metabolomic changes in patients with cDoC is a promising approach and will allow to create prognostic and differential models for this complex category of patients. The next stage of our work will be to determine the structure of the identified compounds, to study their prognostic value and changes depending on circadian rhythm.

There were several limitations in this study. First, the patients received various pharmacological drugs, so we could not exclude their effect on the metabolomic profile. Second, the patients received various types of nutritional support using specialized formulas. The third limitation of the study was the small number of patients and various etiology of brain damage (traumatic brain injury or hypoxia), as well as the absence of a control group of healthy volunteers (due to unavailability of blood sampling from the jugular vein).

Conclusion

Thus, non-targeted metabolomic analysis confirmed the hypothesis of probable significant quantitative and qualitative differences in the composition of metabolites depending on the type of cDoC and the phase of the circadian rhythm. The study identified a set of metabolites which are potential biomarkers for differential diagnosis of VS/UWS and MCS including 4, 33, 6, 1 (in the reverse-phase column experiment) and 14, 35, 41, 48 (in the hydrophilic column experiment), based on their significant contribution to intergroup and intragroup differences. Therefore, the aim of further research is to identify and characterize the above mentioned metabolites.

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Pathogenetic Approach to Early Preeclampsia and the Feasibility of Pregnancy Prolongation

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Summary

Aim. To evaluate the efficacy of cascade plasma filtration (CPF) for the correction of lipid profile and biochemical markers (sFlt-1, PIGF, sFlt-1/PIGF) in pregnant women with early preeclampsia.

Materials and Methods. A prospective controlled study of 23 CPF procedures was conducted in 11 pregnant women with early preeclampsia at gestational ages 22 to 31 weeks. The evolution of clinical manifestations of preeclampsia (BP, urine output, and proteinuria), laboratory biochemical parameters (protein/creatinine ratio, lipid profile), blood coagulation tests, and thromboelastometry (ROTEM) were assessed. In addition, the effect of CPF on the level of preeclampsia markers (sFlt-1, PIGF, sFlt-1/PIGF ratio) as predictors of endothelial aggression was analyzed. The efficacy of extracorporeal therapy was evaluated based on the duration of pregnancy prolongation.

Results. The use of CPF as an adjunct for the treatment of early preeclampsia had a positive effect on the lipid profile by reducing cholesterol and LDL, which helped to decrease atherogenic aggression on the vascular endothelium. In addition, the extracorporeal therapy promoted reduction of the anti-angiogenic effect of sFlt-1, which was confirmed by a significant decrease in the sFlt-1/PIGF ratio from 515 [347; 750] to 378 [285; 557] (P=0.013). The period of prolongation of pregnancy was longer in the main group (with CPF) and was 19 [5; 26] days, whereas in the comparison group (without CPF) it was 3 [1; 4] days (P<0.001). All newborns were discharged from the hospital in a stable condition. The paper is supplemented with a clinical observation of the effective use of CPF in early preeclampsia.

Conclusion. The use of cascade plasma filtration in the treatment of early preeclampsia to prolong pregnancy could be a promising approach.

Keywords: early preeclampsia; cascade plasma filtration; soluble fms-like tyrosine kinase; sFlt-1; vascular endothelial growth factor; VEGF; lipid profile

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Introduction

Developing effective treatments for early preeclampsia is relevant to obstetric practice. Although surveillance, effective diagnosis and early hospitalization have reduced maternal morbidity and mortality, preterm delivery with extremely low fetal weight leads to postnatal complications and high economic costs. Thus, this problem nowadays requires a pathogenetic approach and modern innovative solutions.

Currently, the mechanism of early preeclampsia is associated with impaired remodeling of the spiral arteries and superficial invasion of the cytotrophoblast into the spiral arteries, which leads to placental ischemia and oxidative stress. Subsequently, the altered placenta produces several aggressive factors (sFlt-1, placental endoglin, etc.) that destroy vascular endothelial cells causing endothelial dysfunction. The latter associates with impaired renal blood flow and decreased glomerular filtration rate resulting in increased production of aldosterone and enhanced glomerular sensitivity to angiotensin. All this leads to abnormal water-compartment distribution, sodium retention, as well as increased permeability of the glomeruli to macromolecules, resulting in clinical manifestations of preeclampsia (hypertension, edema and proteinuria) and organ disorders [1, 2]. As early as in 2003, Maynard et al.

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demonstrated that the risk of preeclampsia correlated with increased levels of soluble fms-like tyrosine kinase (sFlt-1) and reduced levels of soluble vascular endothelial growth factor (VEGF). In their studies, the authors created a flexible model of preeclampsia by viral transfection of sFlt-1 in pregnant rats, which subsequently led to the development of hypertension, proteinuria, antenatal fetal death and glomerular endotheliosis [3]. Later, another population-based study demonstrated that sFlt-1 levels raised several weeks before the clinical manifestation of the disease (on average, 3 to 4 weeks), thus predicting the development of preeclampsia [4].

Thus, the theory of endothelial dysfunction in the pathogenesis of preeclampsia is currently considered highly plausible, and previous research confirms the validity of the predictive approach in addressing this problem [5–12]. Effective diagnostic models of preeclampsia prediction have been implemented in everyday practice, and this suggests considering prophylactic methods for the prevention and therapy of this pregnancy complication [13–17].

Based on convincing evidence that increased sFlt-1 blood levels are potentially important in the pathogenesis of preeclampsia, the reduction of serum levels of sFlt-1 and other anti-angiogenic factors has been suggested to inhibit further progression of preeclampsia and prolong pregnancy. In this regard, a pilot international study conducted in 2016 deserves special attention. The authors proposed to reduce sFlt-1 by cascade plasma filtration, or DFPP (double filtration plasmapheresis), using negatively charged dextran-sulfate-cellulose columns (PSDS, Kaneka, Japan). The negatively charged columns were assumed to precipitate positively charged sFlt-1 molecules, which would subsequently reduce their aggressive antiangiogenic effect on the vascular endothelium. Cascade plasma filtration was performed in 11 patients with early preeclampsia diagnosed between 23 and 32 weeks of pregnancy. Maternal and neonatal outcomes were analyzed versus a comparison group of 22 pregnant women with early preeclampsia who did not receive extracorporeal therapy. During the study, the authors obtained encouraging results, achieving an 18% (7–28%) reduction in mean sFlt-1 concentrations. In addition, the cascade plasma filtration was associated with an average reduction of urine P/C (protein-to-creatinine) ratio by 44% (indicating improved glomerular filtration) and a decrease in proteinuria. Ultimately, in pregnant women in the main group, labor was delayed by 7–21 days, while in the comparison group, this prolongation averaged 3 days. Neonatal outcomes were also improved. The duration of lung ventilation was reduced from 11 days in the group without extracorporeal therapy to 2 days in pregnant women receiving apheresis therapy [18].

Advancing the pathogenetic approach to early preeclampsia, we should mention another open pilot study conducted in Germany in 2018. Winkler K. et al. questioned the results of earlier research [19]. The authors of the study suggested that clinically significant changes in the lipid profile occur in early preeclampsia. Even in a normal pregnancy, an atherogenic lipid phenotype has been reported, with an increase in triglycerides, low-density lipoproteins (LDL), and very low-density lipoproteins (VLDL). At the same time, preeclampsia causes more pronounced changes in the lipid profile [21–26]. A recent meta-analysis of 24 case-control studies in 2720 women found that high triglyceride levels correlated with the severity of preeclampsia [27]. This finding was further extended and confirmed in five more cohort studies involving 3147 women in the second trimester before the onset of preeclampsia. Hypertriglyceridemia has been shown to precede the onset of preeclampsia and might be considered a predictor of this pregnancy complication [28]. In addition, a recent study proposed a prognostic model based on serum lipoprotein (a). The authors showed that an increase in this marker of more than 40.5 mg/dL in pregnant women with moderate pre-eclampsia can predict severe pre-eclampsia, while serum lipoprotein (a) level of more than 52.5 mg/dL has high sensitivity and specificity for severe pre-eclampsia [29]. Summarizing the results of previous studies, Winkler K. et al. (2018) suggested that impaired low-density lipoprotein metabolism may contribute to endothelial dysfunction and fetoplacental abnormalities in early preeclampsia. Lipid profile correction was performed using cascade plasma filtration in H.E.L.P. apheresis mode. The treatment was administered to 6 pregnant women with early preeclampsia at 24 to 27 weeks' gestation (main group). In the comparison group (gestational age less than 28 weeks), extracorporeal therapy was not performed. Maternal and neonatal outcomes, the changes in lipid profile, and sFlt-1 and PIGF levels were analyzed. Pregnancy prolongation was 15 days in the main group and 6.3 days in the comparison group (P= 0.027). Triglycerides, cholesterol, LDL, and VLDL levels were reduced by more than 40% in the main group. However, the authors did not reveal a significant decrease in sFlt-1 [19].

Thus, currently, there are two main concepts of using different variants of cascade plasma filtration in early preeclampsia. One concept is aimed at reducing the anti-angiogenic effect of sFlt-1 on vascular endothelium, whereas the another one deals with correcting the atherogenic lipid profile. Both concepts hold validity and prove the effectiveness of extracorporeal therapy [18–20]. However, for a more detailed discussion of the problem and providing a

rationale for practical use of the method, larger randomized studies are required.

Material and Methods

We conducted a prospective comparative controlled study that included all patients admitted from December 1, 2019, to October 31, 2020, to the intensive care unit of Surgut Regional Clinical Center for Maternal and Child Health Care, with a referral diagnosis of early preeclampsia (*n*=28). The criteria for the diagnosis of early preeclampsia were gestational age from 22 to 31 weeks, proteinuria ⩾0.3 g/l, and blood pressure >140 mm Hg. The diagnosis was confirmed if Sflt-1/PIGF ratio > 85 pg/mL (biochemical marker of preeclampsia). This helped rule out the diagnosis of early preeclampsia in 6 patients with Sflt-1/PIGF ratio ⩽ 85 pg/ml (comparison group 1). The remaining 22 patients with Sflt-1/PIGF ratio > 85 pg/ml were randomized into 2 groups which included comparison group 2 (*n*=11) who received only conservative therapy without extracorporeal treatment; 3rd group (main, *n*=11) included patients who received conservative treatment and additional cascade plasma filtration (CPF). All CPF sessions were performed upon the written consent of the patients and after approval by the medical team and the ethical committee. Extracorporeal therapy was offered as an alternative method in the integrated management of preeclampsia, including cases where early operative delivery was rejected.

The CPF procedures were performed on a Plasauto Sigma (Asahi, Japan) machine. The whole cycle of extracorporeal therapy consisted of 2 stages. Stage 1 included separation of patient's blood into cells and plasma using «Plasmaflo» TPE column (Asahi, Japan). Stage 2 consisted of processing of the separated plasma using «Cascadeflo» EC-30W filtration column (Asahi, Japan). The volume of processed plasma during treatment ranged from 1000 to 8000 ml. During the course of extracorporeal therapy, the number of CPF sessions was from 1 to 4. One procedure was done in 5 , 2 in 1, 3 in 4, and 4 procedures were performed in 1 pregnant woman. The interval between CPF sessions was 7 to 12 days. All in all, 11 patients in group 3 underwent 23 CPF sessions. Patients in comparison group 2 (without CPF) received standard therapy in ICU according to the protocol for the management of preeclampsia. Obstetrical strategy and timing of delivery were determined by the changes in clinical and laboratory manifestations of preeclampsia and the antenatal status of the fetus. The participants from group 3 (the main group) had clinical assessment of hemodynamic parameters, urine output, doppler study, and laboratory monitoring before and 1.0–1.5 days after CPF. Clinical and laboratory monitoring included measurement of proteinuria, P/C ratio (urine protein/creatinine), total protein, albumin, cholesterol, triglycerides, LDL and VLDL (AU480 «Beckman Coulter» biochemical analyzer, USA). Coagulation parameters such as fibrinogen, von Willebrand factor (FW), and antithrombin-III were measured using the CS-2000i automatic blood coagulation analyzer «Sysmex», Japan. FW was considered as a relative marker of endothelial dysfunction. In parallel with coagulation system, thromboelastometric parameters (ROTEM delta, «TEM Innovations», Germany) such as clotting time (CT), EXTEM (evaluation of platelet hemostasis), maximum clot firmness (MCF), and FIBTEM (evaluation of plasma hemostasis) were evaluated. The levels of fms-like tyrosine kinase-1 (sFlt-1) and placental growth factor (PIGF) before and after CPF session were determined by electrochemiluminescent immunoassay (Roche, Elecsys sFlt-1/PIGF «Cobas®»). The efficacy of the therapy was assessed based on the duration of pregnancy prolongation and the timing of delivery.

Statistica v.10.0, a standard package of applied statistical analysis software, was used for statistical analysis. The Kolmogorov-Smirnov test showed that variables were not normally distributed, so nonparametric statistical methods were used. The results were presented as *Me* [Q1; Q3]. The specific proportion of a parameter in the total set of data was expressed in percents. Differences between mean values in unrelated samples were compared for significance by the Mann–Whitney method; in related samples, they were compared using the Wilcoxon method. Intergroup comparisons of proportions (in %) were made using the χ^2 method. Correlations between quantitative parameters were examined using the rank correlation method. The detected differences were considered significant at $P<0.05$.

Results

All pregnant women in the main group 3 and comparison group 2 exhibited clinical and laboratory manifestations of preeclampsia (ICD codes O13.0 and O14.0). Two patients in group 2 were diagnosed with severe pre-eclampsia within the partial HELLP syndrome and delivered within 8 to 12 hours of their admission to hospital. All pregnant women with pre-eclampsia who underwent fetal Doppler study (*n*=20) at the time of hospital admission had some manifestation of chronic utero-placental insufficiency (CUPI) and 75% (*n*=15) of them had intrauterine growth retardation syndrome (IGRS). In addition, the diagnosis of preeclampsia was confirmed by high levels of anti-angiogenic markers (Sflt-1 and Sflt-1/PIGF ratio). A comparative analysis

Table 1. Comparison of baseline parameters of the comparison groups.

Parameter	Values			*P* (items 1 and 2 using Mann–Whitney test; item 3 using χ^2)		
	Group 1, *n*=6	Group 2, *n*=11	Group 3, *n*=11	1–2	1–3	2–3
1. Age and gestational age *Me* [Q1; Q3]						
Age, years	24.5 [23; 29]	30 [23; 35]	34 [24; 35]	0.25	0.31	0.34
Gestational age, weeks	31 [28; 31]	30 [28; 31]	29 [23; 30]	0.09	0.22	0.23
2. Laboratory parameters *Me* [Q1; Q3]						
Proteinuria, g/l	0.29 [0.25; 0.72]	2.1 [0.48; 4.77]	1.3 [0.58; 1.72]	0.02	0.06	0.11
P/C-ration	3.0 [2.5; 4.1]	43.5 [12.9; 105.0]	23.9 [14.9; 51.8]	0.03	0.04	0.21
Sflt-1, pg/ml	2169 [1892; 5496]	13264 [6887; 15747]	16069 [10316; 16462]	0.0001	0.0002	0.18
PIGF pg/ml	224 [109; 371]	19 [16; 34]	17 [16; 24]	0.02	0.02	0.15
Sflt-1/PIGF	13 [7; 43]	397 [332; 776]	693 [401; 971]	0.0002	0.0001	0.07
3. Doppler study						
Malperfusion grade 1, *n* (%)	—	5 (45.4)	6 (54.5)	—	—	0.71
Malperfusion grade 2, *n* (%)	—	3 (27.3)	3 (27.3)	—	—	0.23
Malperfusion grade 3, *n* (%)	—	1 (9.0)	2 (18.2)	—	—	0.33
CUPI grade 1, *n* (%)	—	4 (36.4)	5 (45.4)	—	—	0.54
CUPI grade 2, *n* (%)	—	2 (18.2)	3 (27.3)	—	—	0.45
CUPI grade 3, *n* (%)	—	1 (9.0)	—	—	—	—

Note. CUPI — chronic utero-placental insufficiency.

of the laboratory manifestations of preeclampsia and the Doppler results in the comparison groups is presented in Table 1, which shows that the baseline data in Groups 2 and 3 is comparable.

In 8 out of 11 pregnant women with preeclampsia, temporary stabilization of hemodynamic parameters, an increase in urine output, as well as a decrease in proteinuria and P/C ratio were seen after the first CPF session, which suggested an improvement in glomerular filtration. Lipid profile changes in pregnant women with early preeclampsia were particularly noteworthy. Almost all patients had an atherogenic lipid profile (elevated total cholesterol, triglycerides, LDL and VLDL). After CPF, a decrease in all studied lipid profile parameters was observed. In addition, the reduction in total cholesterol and LDL was significant, which confirms the high efficiency of lipoprotein apheresis in correcting the atherogenic profile. The changes in coagulation tests and ROTEM also merit attention. Initially all pregnant women with early preeclampsia had high levels of von Willebrand factor (FW), which was considered as a relative marker of severe endothelial dysfunction. Furthermore, many authors now consider reduced antithrombin III (AT-III) to be a significant and independent criterion of preeclampsia severity, and a decrease in AT-III level in women with hypertension in pregnancy down to the lower limit of normal may be a predictor of early pre-eclampsia development [30–31]. No baseline low AT-III values (less than 70%) were found. Meanwhile, there was a significant decrease of AT-III associated with CPF sessions, which can be explained by extracorporeal clearance. For this reason, AT-III concentrate was administered at a dose of 500–1000 IU in 2 out of 23 clinical cases with an AT-III decrease less than 70%. Assessing the effect of CPF on the blood coagulation system, we conclude that after the extracorporeal therapy the hemostatic potential (as indicated by changes in coagulation tests and thromboelastometry) is reduced. A significant decrease was obtained for the levels of fibrinogen, FW, AT-III, as well as the maximum clot firmness (MCF FIBTEM). Besides, our earlier observations demonstrated loss of protein fractions, primarily albumin, when performing CPF at 40–80% of the circulating plasma volume. Therefore, after each CPF session 100–200 ml 20% albumin was administered. These results can be regarded in two ways. On the one hand, there is an impact of extracorporeal circuit (loss of protein fractions, dilution effect, residual effect of heparin). On the other hand, we cannot rule out the so-called «apheresis component», as the technology of CPF on Plasauto Sigma device implies patient's plasma drainage in order to prolong the work of Cascadeflo filtration column. On average, we conducted one CPF procedure with 150–300 ml of plasma drainage which can be considered as a low-volume plasmapheresis. Analysis of the changes in Sflt-1 and PIGF revealed a positive, although minor, effect of CPF. Only in 5 out of 23 CPF sessions there was an increase in Sflt-1 after extracorporeal therapy. This fact was considered as an additional criterion for early operative delivery. Nevertheless, we obtained a significant decrease in the Sflt-1/PIGF ratio from 515 [347; 750] pg/ml to 378 [285; 557] pg/ml (*P*=0.013). The changes in the laboratory parameters associated with CPF are presented in Table 2.

The efficacy of the extracorporeal therapy was also evaluated based on a comparative analysis of pregnancy prolongation period. All pregnant

Table 2. Changes in laboratory parameters associated with CPF, *Me* [Q1; Q3].

Parameter	Before CPF (*n*=11)	After CPF (*n*=11)	*P*-value (Wilcoxon)
Total cholesterol, mmol/l	6.18 [5.4; 6.7]	3.87 [3.2; 5.1]	0.001
Triglycerides mmol/l	2.77 [2.3; 3.25]	2.48 [2.1; 3.1]	0.36
LDL, mmol/l	3.98 [2.6; 4.4]	2.65 [2.0; 3.2]	0.002
VLDL, mmol/l	1.34 [1.0; 1.6]	1.13 [0.9; 1.4]	0.24
Proteinuria, g/l	1.3 [0.5; 1.7]	0.54 [0.4; 1.3]	0.66
P/C ratio	23.9 [14.9; 51.8]	18.7 [12.6; 46.7]	0.75
Total protein, g/l	55 [50; 57]	50 [47; 54]	0.04
Albumin, g/l	31 [28; 35]	31 [29; 33]	0.75
Fibrinogen, g/l	3.4 [2.8; 4.1]	2.78 [2.6; 3.2]	0.001
FW, %	251 [202; 283]	195 [171; 213]	0.001
Antitrombin III, %	84 [73; 95]	77 [70; 90]	0.007
CT EXTEM, s	60 [55; 67]	62 [58; 72]	0.13
MCF FIBTEM, mm	20 [18; 21]	14 [11; 16]	0.001
Sflt–1, pg/ml	10798 [7984; 16069]	8947 [6652; 12817]	0.21
PIGF, pg/ml	19 [15–27]	21 [16; 27]	0.23
Sflt-1/PIGF ratio	515 [347–750]	378 [285; 557]	0.013

Table 3. Pregnancy outcomes after cascade plasma filtration.

Patient	Gestational age at the time of treatment initiation (weeks, days)	Sflt-1/PIGF*		CPF volume, ml (number of sessions)	Gestational age at the moment delivery (weeks, days)	Prolongation period duration (days)
		Before CPF	After CPF			
A	30 weeks and 4 days	293	208	1000 (1)	34 weeks and 2 days	26
B		971	405	3000 (3)	26 weeks and 1 days	21
C	31 weeks and 1 day	693	871	1200 (1)	31 weeks and 6 days	5
D	23 weeks and 6 days	347	251	3000 (3)	27 weeks and 6 days	28
E	28 weeks and 2 days	1482	1409	1500 (1)	28 weeks and 5 days	3
F	27 weeks	750	268	2500 (2)	29 weeks and 5 days	19
G	22 weeks and 4 days	401	291	4500 (3)	28 weeks	38
H	28 weeks and 2 days	569	913	1800 (1)	28 weeks and 6 days	4
I	27 weeks and 2 days	998	352	8000 (4)	30 weeks	19
K	27 weeks and 4 days	154	285	1500 (1)	28 weeks and 1 day	4
L	25 weeks and 3 days	443	236	6000 (3)	28 weeks and 1 day	19

Note. * — change in Sflt-1/PIGF ratio from the baseline at the moment of treatment initiation until minimal value during the whole course of CPF.

women underwent cesarean section due to clinical and laboratory deterioration of preeclampsia. In two cases, additional manifestations of subcompensated utero-placental insufficiency were observed. The pregnancy prolongation period was longer in the main group and reached 19 [5; 26] days, while in the comparison group it was 3 [1; 4] days (P<0.001). All neonates were discharged from the hospital in a stable condition. The pregnancy outcomes, changes in biochemical markers of preeclampsia (Sflt-1/PIGF ratio), and the main parameters of extracorporeal therapy are presented in Table 3. Achieving a lower Sflt-1/PIGF ratio after CPF sessions was associated with an increase in the pregnancy prolongation period (R=–0.61; P=0.02). We also found a direct and significant relationship between the number of CPF sessions and the duration of pregnancy prolongation period (R=0.61; P=0.01). The results demonstrate the effectiveness of CPF for prolongation of pregnancy in early preeclampsia.

Clinical observation of the use of CPP in early preeclampsia

To illustrate our findings, we present a clinical case on observation of prolongation of early preeclampsia in a patient who underwent 4 CPF procedures.

Patient V., 33 years old, was admitted to Surgut Regional Hospital Center for Maternal and Child Health Care on 04.08.2020 with the diagnosis of 2nd pregnancy at 27 weeks' gestation. Preeclampsia of moderate severity. Fetal breech presentation. Chronic utero-placental insufficiency (grade 1A malperfusion, grade 1 intrauterine growth retardation). Hypertension stage 2, risk 2. Before her admission to the hospital, she received inpatient treatment for preeclampsia in the Nefteyugansk Regional Hospital (methyldopa, nifedipine, magnesium sulfate) with no apparent effect. The systolic blood pressure persisted at 150–160 mm Hg, progressing edema was seen. The patient had a history of full-

Table 4. Changes in lipid profile and preeclampsia markers associated with CPF procedures.

Parameter	Changes in parameters at different gestational ages (weeks, days)							
	27 weeks, 2 days		27 weeks, 6 days		28 weeks, 3 days		29 weeks, 1 day	
	CPF №1		CPF №2		CPF №3		CPF №4	
	before	after	before	after	before	after	before	after
Total cholesterol, mmol/l	6.6	3.7	—	3.31	—	3.04	—	2.78
Triglycerides, mmol/l	2.47	2.36	—	1.91	—	1.88	—	2.1
LDL, mmol/l	4.47	2.62	—	2.09	—	2.01	—	2.29
VLDL, mmol/l	1.12	1.07	—	0.87	—	0.84	—	0.95
Sflt-1, pg/ml	16462	14529	11546	9625	11016	10809	12683	12817
PIGF, pg/ml	16.49	28.5	21.23	27.3	19.7	21.6	25.4	29.86
Sflt-1/PIGF ratio	998	509	543	352	559	500	495	429

term delivery in 2018 (fetal weight was 2,700 g). On admission, the patient complained of headache, severe edema (+++) and hypertension up to 140/80–150/90 mm Hg. On laboratory examination, proteinuria was 0.35 g/l (daily protein loss was 1.5 g/l), serum ALT was 68 units, AST was 66 units, total protein was 57 g/l. Other biochemical parameters were normal, coagulation studies were normal for the gestational age. Atherogenic lipid profile was found (increased cholesterol, triglycerides, LDL and VLDL). Atherogenic index of plasma was 3.68 (normal reference 2.0–3.0). The markers of preeclampsia were were measured: Sflt-1 was 16462 pg/ml, PIGF was 16.49 pg/ml, and Sflt-1/PIGF ratio was 998. Treatment with antihypertensives and magnesium sulfate in the ICU for 2 days was not effective, hypertension and edema persisted. Due to the obstetric status, chronic utero-placental insufficiency, and the lack of treatment efficacy, after a course of dexamethasone 24 mg/day to prevent fetal respiratory distress, the patient was offered an early surgical delivery at 27 weeks' gestation + 2 days, which she refused. In order to prolong pregnancy and as an adjunct to the integrated therapy of preeclampsia, four CPF sessions were performed. A total of 8000 ml of plasma (2,000 ml per one session of CPF) were processed during the entire course of extracorporeal therapy. The changes in lipid profile and preeclampsia markers of the patient are presented in Table 4.

Early operative delivery was performed on August 25, 2020, at 30 weeks' gestation due to the lack of treatment effect (persistent hypertension despite using 3 antihypertensive drugs, daily protein loss 0.41–1.5 g/l) and worsening of Doppler parameters (chronic utero-placental insufficiency with 3 grade malperfusion, 2 grade intrauterine growth retardation). A female fetus was born weighing 800 g with Apgar score of 6–7. The newborn stayed in the intensive care units for 82 days and was discharged in stable condition. Thus, the period of pregnancy prolongation was 19 days from the initiation of extracorporeal therapy.

Conclusion

The addition of cascade plasma filtration to the treatment of early preeclampsia is a promising approach to prolong pregnancy.

Cascade plasma filtration is effective for lipid profile correction (total cholesterol, triglycerides, LDL, VLDL).

Cascade plasma filtration as a part of the complex treatment of early preeclampsia reduces sFlt-1 and sFlt-1/PIGF ratio and helps to prolong pregnancy by 19 [5; 26] days.

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Selective Brain Hypothermia in the Comprehensive Rehabilitation of Patients with Chronic Consciousness Disorders

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Summary

Aim: to evaluate clinical effectiveness of selective hypothermia of cerebral cortex for the recovery of awareness in patients with chronic disorders of consciousness (CDC).

Material and methods. 111 patients with CDC 30 and more days after a cerebral event (ischemic or hemorrhagic stroke, brain injury) were included in the study. Exclusion criteria were anoxic brain injury (sequelae of a prolonged asystole or asphyxia), active sepsis, arrhythmia, baseline hypothermia (body temperature lower than 35.5°C). Experimental group included 60 patients, of them 39 patients were in a vegetative state (VS), 21 patients exhibited patterns of minimally conscious state (MCS). Control group incluted 51 patients, of them 32 patients were in VS and 19 patients were in MCS. Patients in the experimental group received 10 sessions (120 minutes each) of selective brain hypothermia (SBH) during the 14-days follow-up period. Patients of both groups received standard identical neurological treatment and rehabilitation procedures. Patients in the control group did not undergo brain hypothermia. The induction of SBH involved cooling of the whole surface of the craniocerebral area of scalp using special helmets. The temperature of the internal surface of the helmet was 3–7°C. Temperature of the frontal lobes of the cortex was monitored with non-invasive microwave radiothermometry, axillary temperature was also registered. The level of consciousness was evaluated using «Coma Recovery Scale-Revised» (CRS-R) scale.

Results. 120-minutes long SBH session reduced the temperature of the frontal lobes of the cerebral cortex by 2.4–3.1°C with no impact on the axillary temperature. Evaluation using CRS-R revealed improvement in all studied functions (auditory, visual, motor, oromotor, communication, arousal) in patients in the experimental group after 10 SBH sessions. Level of consciousness in patients from the experimental group in VS increased from 4.5±0.33 to 8.7±0.91 points (P<0.001), for patients in MCS from 11.3±1.0 to 18.2±0.70 (P<0.001) points. In the control group, scores of patients in VS rose from 4.3±0.37 to 6.8±0.49 (P<0.001) points with the most significant changes in auditory and visual functions (P<0.001). In the control group of patients in MCS the oromotor function improved (P<0.05), overall CRS-R scores changed insignificantly from 9.1±0.57 to 10.1±0.86 (P<0.1). The best outcome (CRS-R>19 points) was seen in patients from the experimental group [6 in VS (15.4%) and 8 in MCS (31.8%)]. In the control group, the best results did not exceed 10 points for the patents in VS, while 4 patients in MCS (21%) reached 12–16 scores. During 30-day follow-up period of hospitalization after the SBH sessions mortality rate was 10% (6 patients) in the experimental group and 21.6% (11 patients) in the control group.

Conclusion. Patients with CDC could benefit from serial SBH sessions performed as a part of comprehensive treatment and rehabilitation strategy. We suggest that selective reduction of frontal lobe temperature improves neurogenesis, neuronal regeneration, and neuroplasticity.

Keywords: hypothermia; frontal lobes; chronic disorders of consciousness; neuroprotection; heat shock proteins; cold shock proteins

Conflict of interest. The authors declare no conflict of interest.

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Introduction

Significant advances in modern critical and intensive care have produced a significant increase in the number of patients with severe brain damage who evolve from coma to other chronic disorders of consciousness (CDC), which include vegetative state (VS) and minimally conscious state (MCS) of undetermined duration [1–3].

Neurological examination still predominates in the diagnosis of VS or MCS, despite a wide range of available advanced diagnostic methods. Its reliability can be increased by using clinical scores including the Coma Recovery Scale — Revised (CRS-R. 2004) which helps reveal and document the first manifestations of consciousness and differentiate between VS and MCS [4, 5].

To date, no generally accepted guidelines and standards for diagnosis, outcome prediction, therapy and rehabilitation principles for this category of patients have been developed. Understanding of the pathogenetic mechanisms of reduced consciousness in brain damage and its recovery after coma is still lacking [6–8].

The treatment of patients with CDC includes maintenance of vital functions, while the rehabilitation measures are focused on the restoration of central nervous system activity, and the choice of rehabilitation techniques depends on their availability in a particular clinic, the patient's tolerability and their response to the procedures [9, 10]. The use of various pharmacological agents aimed at increasing the level of consciousness does not provide a sustainable positive result, and symptomatic therapy dominates in the treatment and rehabilitation of patients with CDC [11].

The study of endogenous cytoprotection and organ protection mechanisms developing as a generic nonspecific response on exposure to potentially damaging factors has become one of the popular areas of neuroprotection and neurorehabilitation research. In particular, the study of ischemic preconditioning (IP) and controlled hypothermia revealed distinct organoprotective effects inherent in almost all internal organs and the brain [12]. However, IP has not been fully adapted for clinical use, since ischemic tolerance of the organs develops when subterminal intensity (potentially dangerous) ischemia episodes are reproduced, whereas there are no effective ways for reliable assessment of ischemic load.

The high neuroprotective properties of hypothermia, associated with metabolic depression and genomic response of cells to reduced temperatures, make it attractive for clinical use in brain damage. However, current therapeutic hypothermia (TH) techniques in most cases employ general cooling of the patient with a decrease in body temperature to 32–33°C, which associates with various side effects and complications. The use of target temperature management (TTM) in cerebral accidents aims to control fever, but not neuroprotection [13].

Meanwhile, the use of selective cortical hypothermia (SCH) achieved by craniocerebral hypothermia (CCH) could provide reduction of brain surface temperature down to the level of local hypothermia required for the expression of cytoprotective genes. We use the term SCH rather than CCH, as it more accurately reflects the target of hypothermia, which is the hemisphere cortex. This cooling technique manages to lower the temperature of the brain surface only, with little effect on the temperature of basal structures and the body [14]. The feasibility of craniocerebral cooling for developing neuroprotection has been clearly demonstrated in experiments and clinical setting [15, 16].

The use of hypothermia, including SCH, involves monitoring the temperature of the tissues being cooled. Recently, the noninvasive measurement of the cortical temperature based on recording the power of brain's own electromagnetic radiation in the UHF range (3–7 GHz) has been introduced in the diagnosis of cerebral damage [17].

Assuming that SCH is capable of lowering the temperature of the hemisphere cortex and triggering neuroprotective response [18], we conducted a pilot study of the effectiveness of this method under cortical temperature monitoring using UHF radiothermometry in patients with CDC.

The aim of the study was to assess the feasibility of clinical use of selective hemisphere cortical hypothermia (SHCH) in patients with chronic disorders of consciousness (CDC).

Material and Methods

The study included 111 patients with CDC. Inclusion criteria were brain conditions following severe focal brain damage (post ischemic or hemorrhagic strokes, severe traumatic brain injury) at least 30–45 days after cerebral accidents and recovery from coma. Exclusion criteria were anoxic brain damage (after prolonged asystole or asphyxia) with widespread diffuse damage to the cerebral cortex, sepsis, cardiac rhythm disorders, baseline hypothermia (body temperature below 35.5°C), terminal illness.

All patients were randomized into two groups. The main group (n=60) included two subgroups. The first subgroup (M1) was composed of the 39 patients in VS (15 women with the mean age 36.7±4.4 years, 24 men with the mean age 43.3±3.4 years). The second subgroup (M2) included 21 patients with MCS minus (7 women with the mean age 44.6±7.7 years and 14 men with the mean age 47.5±3.2 years). The comparison group (n=51) also

included two subgroups. The first subgroup (C1) was composed of of 32 patients in VS (20 women with the mean age 46.9±3.2 years, 12 men with the mean age 44.1±4.1 years). The second subgroup (C2) comprised 19 patients in MCS-minus (10 women with the mean age 56.1±3.5 years and 9 men with the mean age 49.2±3.0 years).

In both groups, the results were recorded on day 1 and 14 of follow-up. After 30 days, mortality was recorded in both groups.

In both groups patients received standard neurotropic therapy and rehabilitation including correction of vital signs, gradual weaning, swallowing correction and removal of tubes, massage, physical therapy, verticalization, myoelectrostimulation, magnetic stimulation, speech therapy, and neuropsychological support.

Patients in the main group received 10 SHCH sessions of 120 minutes duration during the 14-day follow-up period. Patients in the comparison group did not undergo SHCH.

The ATG-01 (Kalashnikov, Russia) therapeutic hypothermia machine was used for the induction of SHCH. The entire surface of the craniocerebral region of the head was cooled using cryoapplicator helmets with the inner temperature of 3–7°C. The cooling procedure was completed by removing the helmet, followed by rapid spontaneous warming of the large hemisphere cortex in the patients. The temperature of the frontal cortex during cooling and the body temperature were monitored.

The cooling modes were chosen empirically based on data from noninvasive UHF radiothermometry of the brain, which allowed monitoring the level of cortical temperature decrease.

UHF radiothermometry of the hemisphere cortex was performed using the RTM-01-RES device (RES LLC, Russia). The device allows to register the power of brain's own electromagnetic radiation at the depth of 4–5 cm from the scalp surface and measure the brain surface temperature in Celcius degrees, as the radiation power is proportional to the intensity of tissue metabolism and its temperature. Measurements were taken in the projection of the left and right frontal lobes using an antenna placed on the scalp directly next to the cooling helmet (Fig. 1). The temperature was measured prior to the procedure and then every 30 min until the end of cooling, immediately after the cooling, and 30 min later. The studies were performed in standard conditions of the intensive care unit (ambient temperature 25–27°C, humidity 75–80%).

The level of consciousness was assessed according to the Coma Recovery Scale-Revised (CRS-R, 2004) with evaluation of auditory, visual, motor, oromotor, communication and arousal functions (in points). CRS-R scale results of the main group patients before the first session and on day 14 after the 10th session were analyzed. In comparison group patients, CRS-R scale data were taken on the day of enrollment and on day 14.

Fig. 1. Temperature measurement.
Note. The antenna is placed over the projection of the left frontal lobe.

The significance of the obtained data was confirmed based on:

- Sufficient sample size for a pilot study (111 patients were divided into the main and comparison groups, each of which had an adequate number of patients allowing for statistical analysis)
- Statistical analysis of results using the SPSS Statistics 21.0 software package. To assess the significance of differences between the groups, the Student's t-test was used, with a prior estimation of distribution of variables for normality. Differences were considered significant at $P \leqslant 0.05$.

Results

Temperature measurements showed that before the first and next procedures, the mean temperature in the projection area of the frontal lobes of the left (LH) and right (RH) hemispheres did not differ (36.4±0.1°C and 36.4±0.1°C, respectively) between the VS and MCS-minus groups. Body temperature was 36.4±0.1°C. After 30 minutes of cooling, the LH and RH temperatures began to drop, and after 90 minutes were 33.9±0.4°C and 33.5±0.5°C, respectively. After removing the cooling helmet from the patient's head, the temperature in the LH and RH was 34.0±0.4°C and 33.3±0.5°C and remained low after 30 minutes post cooling at 35.7±0.1°C and 35.7±0.1°C, respectively. Throughout the cooling period and after the procedure, the body temperature did not alter, remaining within the normal limits (Fig. 2).

The total CRS-R score was 6.9±0.6 in the main group (n=60), 4.5±0.3 in the M1 subgroup (VS, n=39), and 11.3±1.0 in the M2 subgroup (MCS,

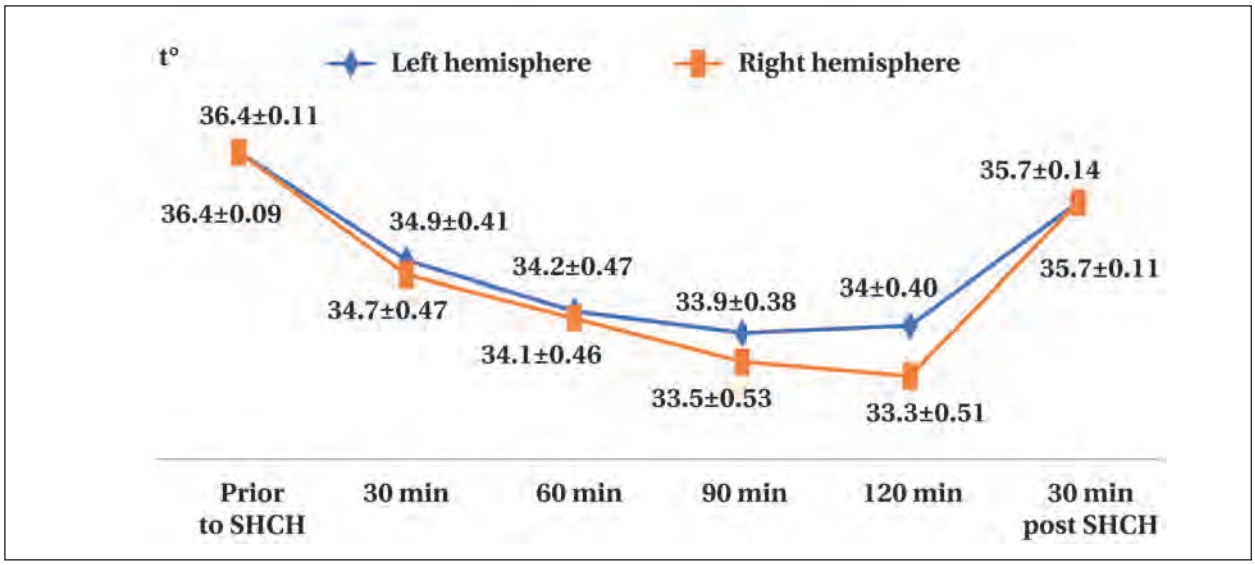

Fig. 2. Evolution of temperature changes in the left and right frontal lobes before and after SHCH.
Note. t° — mean temperature of frontal lobes. 30 min, 60 min, 90 min, 120 min — temperature readings in the frontal lobes at time points during the cooling session.

n=21) on day 1 of the study. The total CRS-R score was 6.1±0.5 in the comparison group (*n*=51), 4.3±0.4 in the C1 subgroup (VS, *n*=32), and 9.1±0.57 in the C2 subgroup (MCS, *n*=19).

On day 14 of the study, after the 10th SHCH procedure, the CRS-R score in the main group was 12.1±0.9, demonstrating a highly significant increase (*P*<0.001). In the comparison group, the CRS-R score on day 14 of the study was 8.1±0.5 which was also significant (*P*<0.05). In the main group, this score reached 8.7±0.91 in the M1 subgroup (*P*<0.001) and 6.8±0.49 in the C1 subgroup also on day 14 (*P*<0.001). In the M1 subgroup, auditory, visual, oromotor, communication, and arousal functions increased most significantly (*P*<0.001), and motor function increased slightly less significantly (*P*<0.005). In the C1 subgroup, only auditory and visual functions increased most significantly (*P*<0.001), while motor, oromotor, and communication functions increased less significantly (*P*<0.005) and the level of arousal function remained unchanged.

The averaged data show that patients in VS who received the SHCH course reached the CMC-minus level, whereas the changes in the CRS-R scale were less evident in the C1 subgroup.

Reflecting the general trend of increasing level of consciousness in patients in both groups and in each subgroup, the average values do not take into account heterogeneity of the results. Thus, in the M1 subgroup, the best results (CRS-R >16 points) were obtained in 6 patients (15.4%): 3 patients reached 16–19 points (MCS-plus), and CRS-R values reached 20–21 points in another 3 patients, indicating their progression to clear consciousness. In the C1 comparison group (SHCH not performed), the best results (CRS-R> 11–13 points), were achieved in 5 patients (15.6%), which corresponds to the CMS-minus level.

Functional assessment using the CRS-R scale in patients of the M2 and C2 on day 1 of the study showed that they were in MCS-minus. On day 14 of the study, after the SHCH course, the CRS-R score for all studied functions in the M2 subgroup demonstrated significant increase reaching 18.2±0.7 points (*P*<0.001). Patients in the comparison group (C2 subgroup) also showed an increase of oromotor function on day 14 of follow-up (*P*<0.05), but the mean increase of CRS-R score to 10.1±0.86 points was not significant (*P*>0.1).

Heterogeneity of results after 14 days was observed in both subgroups of the main group and the comparison group. In the main group, in the M2 subgroup, the best results (CRS-R > 16 points, MCS-plus) were obtained in 8 patients (38.1%), and in 5 patients in this group, CRS-R values reached 20–23 points, indicating a significant recovery of consciousness. In C2 comparison group (no CRS-R was assessed), 4 patients (21%) achieved a CRS-R score of 12–16 on day 14, which corresponded to MCS-plus.

The functional changes on the CRS-R scale in patients of the main and comparison subgroups are shown in the table below.

Analysis of mortality after 30 days showed that in the main group 6 patients of the M1 subgroup died (15.4%). In the M2 subgroup, all patients were alive. In the comparison group, 7 patients died in the C1 subgroup (21.9%) and 4 patients died in the

The functional changes on the CRS-R scale in patients of the main and comparison subgroups

Functions according to CRS-R	Parameter values in groups							
	Main (SHCH)				Comparison			
	M1		M2		C1		C2	
	Day 1	Day 14	Day 1	Day 14	Day 1	Day 14	Day 1	Day 14
Auditory	0.7±0.10	1.5±0.18***	2.2±0.23	3.3±0.12***	0.7±0.11	1.3±0.11***	1.6±0.16	1.5±0.19
Visual	0.8±0.11	1.9±0.23***	2.6±0.31	4.1±0.22***	0.8±0.10	1.3±0.10***	1.8±0.16	2.1±0.21
Motor	1.3±0.13	2.1±0.24**	3.1±0.31	4.8±0.19***	1.2±0.15	1.7±0.11**	2.3±0.18	2.4±0.27
Oromotor	0.4±0.09	0.9±0.13***	0.8±0.15	1.8±0.17***	0.2±0.07	0.6±0.12**	0.7±0.15	1.1±0.17*
Communication	0.1±0.04	0.6±0.11***	0.6±0.15	1.5±0.11***	0.2±0.07	0.5±0.12**	0.9±0.15	1.0±0.20
Arousal	1.3±0.11	1.8±0.14***	2.1±0.16	2.8±0.12***	1.3±0.12	1.5±0.13	1.8±0.16	2.0±0.13
Total	4.5±0.33	8.7±0.91***	11.3±1.0	18.2±0.70***	4.3±0.37	6.8±0.49***	9.1±0.57	10.1±0.86

Note. * — $P \leqslant 0{,}05$; ** — $P \leqslant 0{,}01$; *** — $P \leqslant 0{,}001$. The M1 and C1 patients were in vegetative state, the M2 and C2 patients were in minimally conscious state.

C2 subgroup (21.1%). A total of 6 patients (10%) died in the main group and 11 patients (21.6%) in the comparison group. The main causes of death in both groups were infections, thromboembolic complications, multiple organ failure.

No complications or side effects of SHCH were registered, and the patients tolerated the procedures well.

Discussion

The mechanisms of neuroprotective effects of hypothermia during the acute period of brain disorders have been extensively studied [19–21]. They include metabolic reactions such as limited consumption of oxygen and substrate, inhibited excitotoxicity reactions and receptor-mediated interactions of signaling molecules, control of edema and inflammatory response, apoptosis, etc. Meanwhile, the temperature signal appearing within the range of 2–3°C variation is sufficient for the expression of genes encoding a wide array of different stress-protecting proteins.

The expression of early genes c-fos and c-jun has been shown to develop when decreasing temperature in cortical neuronal culture leads to accumulation of various cold shock proteins (CSPs) [22]. Temperature fluctuations within 1–3°C are sufficient for expression of genes encoding the synthesis of heat shock proteins (HSPs). However, temperature increase promotes a decrease in HSP production, while warming induces an increase in their production even at lower temperatures (below 32°C). These data were confirmed in many experimental studies [23].

HSPs and CSPs are reasonably considered to be stress proteins with a high potential of neuroprotection, and the initiation of their production is associated not only with temperature signal, but also with exposure to other potentially dangerous stimuli [24]. The studied class of stress-proteins promoting neuroprotection and activating neuroregeneration and neuroplasticity is quite extensive [25, 26]. Importantly, the effects of early gene expression persist for up to several days.

The above-mentioned prerequisites allowed assuming that a course of daily SHCH procedures providing a 2.5–3°C reduction in the brain surface temperature can result in the accumulation of stress proteins, which, theoretically, can have a positive effect on the recovery of consciousness in patients with CDC. These assumptions have been supported, to a certain extent, by the results of clinical studies.

Use of a hypothermic effect on the brain in patients with CDC resulting from severe brain damage and manifesting as VS and MCS-minus does not seem to be as self-evident as in acute brain conditions. However, it is necessary to keep in mind that the completed damage pattern seems to be mostly applicable for the destructive events which had already occurred and cannot be corrected. Meanwhile, the patients with CDC still retain a certain rehabilitative potential which determines the outcome of the disease. The rehabilitation strategy in these cases is aimed at using methods activating body's own resources and increasing the rehabilitation potential. The latter include various neuromodulation methods such as transcranial magnetic and electrical stimulation, as well as SHCH. In particular, a 120-minute cooling period has been shown to provide a 15–20% decrease in the linear blood flow rate in the major cerebral vessels (anterior, middle and posterior cerebral arteries), and during the warming period the blood flow rates quickly return to their initial values [15]. That is, a period of non-hazardous hypoperfusion develops when the temperature decreases, and reperfusion initiates upon warming. Active radicals, as well as the temperature reduction itself, become a powerful trigger for the expression of early genes encoding stress proteins, which could prevent the progression of destructive processes and increase the potential of intact brain areas.

These hypotheses are rather speculative, but the results of our pilot study demonstrate the positive impact of SHCH in patients with CDC, which

emphasizes the feasibility of this approach in an integrated rehabilitation plan. Obviously, further in-depth studies of selective brain hypothermia are needed to improve the effectiveness of therapy and rehabilitation of CDC patients. Determination of molecular markers of brain damage and recovery as well as of the oxidative status seems essential for clarification of mechanisms of the obtained effects.

Conclusion

Our results demonstrate a positive effect of selective brain hemisphere hypothermia on the recovery of consciousness in patients with CDC. The use of SHCH courses in patients with CDC can be recommended as part of an integrated treatment and rehabilitation plan. Selective hypothermia of brain hemispheres could improve neurogenesis, neuroregeneration and neuroplasticity.

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Changes in Resting fMRI Networks in Patients with Severe Traumatic Brain Injury During Therapeutic Rhythmic Transcranial Magnetic Stimulation (Case Report)

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Summary

Severe traumatic brain injury (TBI) accompanied by impaired consciousness manifesting as prolonged postcoma unawareness (PCU) is one of the current medical and social problems causing high morbidity and mortality worldwide. Difficult recovery of such patients necessitates the development of additional neurorehabilitation approaches, including neuromodulation methods, as well as the search for objective markers of treatment efficacy.

Aim of the study: to evaluate the effect of therapeutic rhythmic transcranial magnetic stimulation (rTMS) on fMRI resting state networks (RSN) in PCU after severe TBI.

Materials and Methods. We analyzed individual fMRI RSN in three patients with PCU before and after a course of rTMS performed at different timepoints after severe TBI and with different efficacy of treatment. We assessed the topography and quantitative characteristics of the networks (DMN, sensorimotor, control functions, left and right fronto-parietal, auditory, and speech) known to be most significant for recovery of consciousness.

Results. We found a trend toward normalization of RSN topography as well as an increase in the integral index of network intensity in two of three patients with a distinct increase in consciousness after a course of rTMS.

Conclusion. Using case observations, we have demonstrated the therapeutic efficacy of rTMS and feasibility of using fMRI RSN as a reliable diagnostic approach in PCU following severe TBI.

Keywords: traumatic brain injury; unawareness; resting state networks; fMRI; rTMS

Conflict of interest. The authors declare no conflict of interest.

Full text of the article in Eng. on www.reanimatology.com

Introduction

Severe traumatic brain injury (STBI) associated with impaired consciousness and motor activity is one of the urgent medical and social issues causing high morbidity and mortality worldwide [1, 2]. Consciousness disorders such as coma and a high risk of prolonged (chronic) post-coma unawareness (PCU) are characteristic of STBI [3, 4]. Meanwhile, the assessment of cognitive functions and recovery potential for mental activity in general plays a crucial role in the integrated treatment and neurorehabilitation strategy.

PCU includes phases immediately following coma and succeeding each other, beginning with the first post-coma opening of the patient's eyes and ending with the reappearance of contact with him/her (most often as a clear compliance with the instructions) [3, 5–7]. Several clinical scales are

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used for quantitative assessment of PCU, and their relevance is determined by current perspectives and the research progress [7–10].

To date, several international guidelines for the treatment of this category of patients have been published [7, 11]. However, significant difficulties of patient rehabilitation provide a rationale for the search and development of additional neurorehabilitation approaches, which include neuromodulation methods [4]. Transcranial electrical and magnetic stimulation has been used in clinical practice for the past decade as a neuromodulatory intervention [12, 13]. The therapeutic use of rTMS is based on its ability to change the level of cortical excitability, causing depolarization of neurons with subsequent generation and propagation of action potential (AP) [14, 15]. Changes in hemodynamics, neurotrophic factor production, and neurotransmitter activity associated with rTMS have also been shown [13].

The use of rTMS seems very promising for the neurorehabilitation of patients with disorders of consciousness and motor function following STBI [16]. According to the literature, as well as our own research, stimulation of frontal hemispheres (zones Fz, F3–F4 or F3 according to the international scheme of electrode positioning 10–20% in electroencephalography) has significant positive effect [17–19]. The effectiveness of stimulation of these areas can be explained, among other things, by the fact that the frontal cortical areas are considered to be pivotal in providing the so-called executive functions that initiate, plan, regulate and control any purposeful activity [20, 21].

On the other hand, the rather complex mechanism of action of rTMS, especially in variable conditions of brain damage, determines possible individual differences in the brain responses of patients with a traumatic brain injury to this therapeutic intervention. The timing of rTMS integration into neurorehabilitation, in particular, in early period following traumatic brain injury during intensive therapy, is one of the controversial issues in this area. The study of systemic cerebral reactions, accompanying rTMS, as well as control of the effectiveness of stimulation necessitates the involvement of most informative parameters of brain performance.

The concepts of neural network structural and functional organization of brain activity [22], as well as the analysis of fMRI resting state networks [23] are currently widespread in neuroscience. The formation of resting state networks (RSN) is associated with high correlation in time of interregional hemodynamic signals [24]. There have been described 7 to 15 functional RSNs that are most typical for healthy people [23, 25], but are impaired in brain conditions, including traumatic brain injury [26]. A number of RSNs are considered in the literature to be the most significant for recovery of consciousness in PCU. They include such networks as default mode network (or DMN), executive functions, lateral frontoparietal, auditory, sensorimotor, visual, salience [27, 28], and probably speech network [29, 30] (Fig. 1).

In a series of our previous studies, we developed an algorithm for group and individual analysis of fMRI resting state networks (RSN) based on the ICA FSL software [31]. We have shown its informative value for the estimation of RSN in intact brain and in STBI [31, 32].

This study aims to investigate changes in the network organization of the brain in patients with STBI, accompanied by disorders of consciousness and motor activity, during therapeutic rTMS. The main focus was placed on RSNs shown in Fig. 1.

The aim was to evaluate the effect of therapeutic rhythmic transcranial magnetic stimulation (rTMS) on fMRI resting state networks (RSN) in PCU after STBI.

We focused on the determination of general trends in fMRI RSN changes in patients with STBI exposed to rTMS and identification of patterns of fMRI RSN associated with clinical effect of rTMS.

Material and Methods

We studied three patients (two men and one woman) who had suffered a traumatic brain injury resulting in post-coma unawareness state (Table 1). The current clinical status and level of consciousness were assessed using the CRS-R scale [9] as well as the consciousness recovery assessment scale [33]. According to this assessment, at the time of the 1st examination, one patient was in the unresponsive wakefulness syndrome (UWS) or vegetative state, two patients were in the MCS- or akinetic mutism (arbitrary fixation of gaze without executing instructions and speech production). Motor defect manifested as hemiparesis was assessed using a muscle strength scale [34]. All patients were not on ventilatory or oxygen support.

The current lack of generally accepted standards for therapeutic rTMS in patients with TBI [13] warrants the search for new and improved stimulation algorithms. In this study, we used a protocol developed earlier [19]. Stimulation was performed on a MagPro×100 device (MagVenture) using the 'figure-of-eight' B-70 coil. Each patient underwent a course of rTMS on zones F3 and F4 according to the 10–20% EEG system (left and right dorsolateral prefrontal cortex, respectively). The duration of the TMS course varied from 5 to 10 sessions. One rTMS session included from 1000 to 4000 pulses with 50% maximum stimulator power (MSP). The threshold was determined according to the clinically accepted technique of diagnostic rTMS performed before the

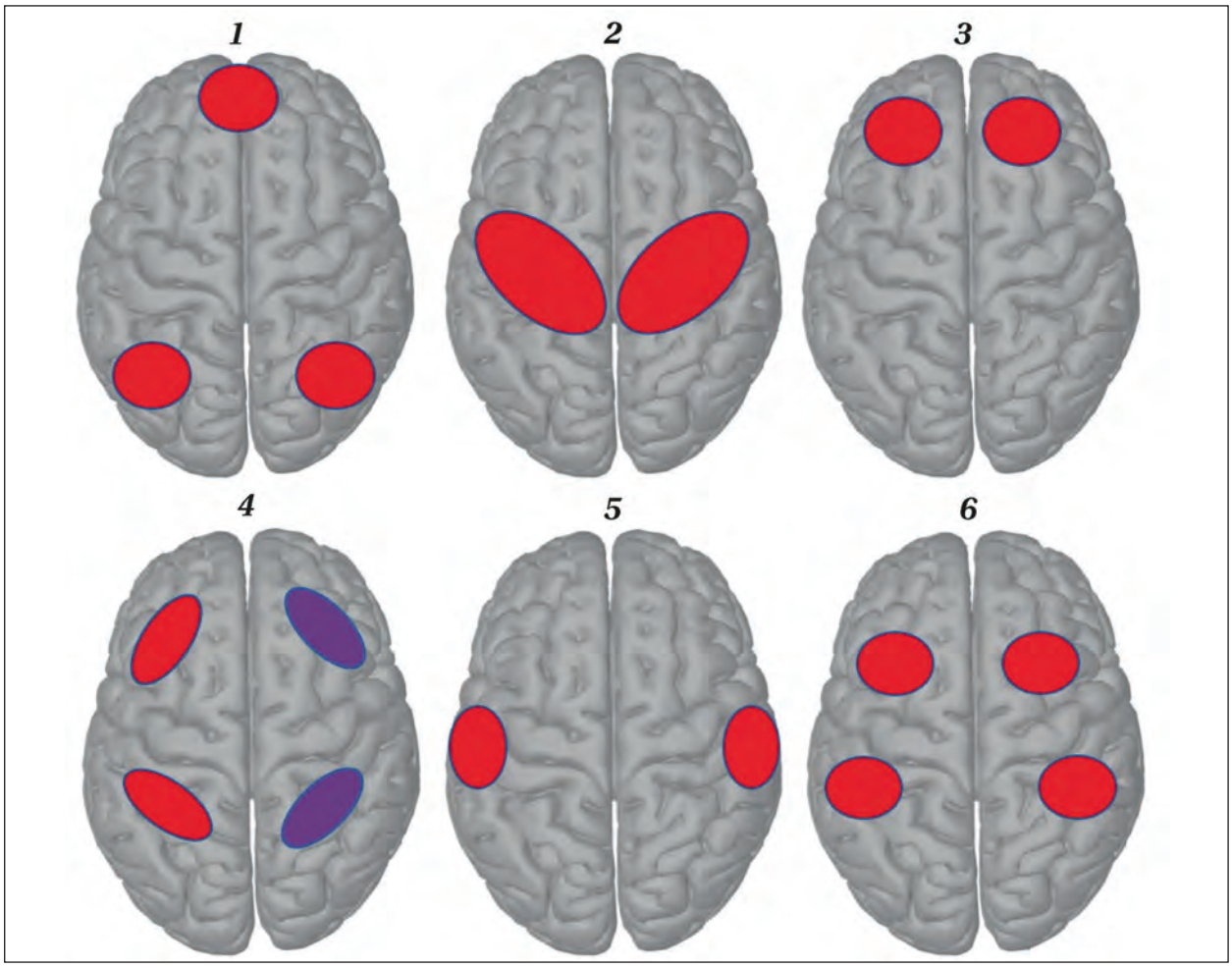

Fig. 1. Schematic representation of hemispheric projections of resting fMRI networks considered significant for recovery of reduced consciousness.
Note. *1* — DMN; *2* — sensorimotor network; *3* — executive functions; *4* — frontoparietal (left and right); *5* — auditory; *6* — speech.

Table 1. Characteristics of patients' level of consciousness before and after a course of rTMS.

Patient	Parameters							
	Sex	Age	Time after trauma	Post rTMS		Number of rTMS sessions	Post rTMS	
				On consciousness recovery assessment scale	On CRS-R		On consciousness recovery assessment scale	On CRS-R
M.	M	34	17 days	VS	UWS	5	AM-MSU	MCS+
B.	F	35	17 months	AM	MCS-	6	MSU	MCS+
Ya.	M	31	4.5 years	AM	MCS-	10	AM	MCS-

Notes. UWS — unresponsive wakefulness syndrome; MCS- — minimally conscious state without following commands; MCS+ — minimally conscious state able to follow commands; VS — vegetative state; AM — akinetic mutism; MSU — mutism with speech understanding.

course stimulation including bilateral stimulation of the motor cortex (M1) and neck region (CVII). The range of rTMS frequencies, which varied from 1 to 10 Hz, was selected for each patient individually depending on the lesion area anatomy and performance changes assessed using clinical examination and EEG data. Stimulation was performed along with a personalized, continuous and invariable treatment regimen, which included anticoagulants/antiaggregants, neurometabolic and gastroprotective drugs, as well as prophylactic doses of anticonvulsants.

After a course of rTMS, we recorded a qualitative improvement in two patients and no changes in the clinical condition were observed in one patient (Table 1).

Before and after the course of rTMS the fMRI was recorded using a General Electric Signa HDxt

magnetic resonance tomograph (USA) at rest with eyes closed for 10 min and 12 sec in the Department of radiology and radioisotope diagnostics of the Burdenko Scientific Research Center of the Russian Ministry of Health.

A 3D FSPGR pulse sequence (BRAVO) was used to obtain structural data (whole brain volume) with the following parameters: TR = 8.8 ms, TE = 3.5 ms, slice thickness = 1 mm, FOV = 250 mm, image matrix 256×256, voxel size 0.97×0.97×1.0 mm. The echoplanar sequence Spin Echo (BOLD T2) was used to obtain functional data. TR = 2000 ms, TE = 30 ms, slice thickness = 3 mm, FOV 250 mm, image matrix 128×128, voxel size 1.95×1.95×3 mm. In each time series, 300 sets of functional volumes were obtained, each containing 24–40 axial slices capturing the entire brain. Scanning time per functional volume unit was 2 seconds. The total number of slices in a functional series was 7000–12000.

During fMRI registration the primary control of the quality of hemodynamic signals, automatic noise correction, as well as assessment of the quality of block recordings by the presence of motor artifacts (excellent, good, poor) were performed. In the latter case, the scanning was interrupted, and the study was started again. All studies were performed without anesthesia.

The output experimental data were recorded in DICOM format with subsequent conversion to NIFTI and processing in the FMRIB Software Library (FSL) [31].

Processing included removal of artifacts associated with low-frequency noise and correction of motion artifacts, then conversion of functional data to standard space (anatomical structure of the brain), and then, using the MELODIC-ICA tool, analysis of fMRI RS.

A series of special studies established the feasibility of limiting the number of networks at 60. With N > 60 or no limitation, the major RSNs normally became fragmented.

The data obtained were superimposed on individual brain images in axial, frontal, and sagittal planes.

As a result, several voxel groups (large-scale networks) with statistically independent BOLD-signal changes were distinguished in each patient. The significance level for the selection of independent components was $P<0.01$. Correction for multiple comparisons was performed automatically when the p value was lower than 0.05. The selected networks were then visualized in the three-dimensional image of the brain of each patient.

Three independent experts in neurophysiology, radiology, neurology participated in the verification of individual RSNs of patients, taking into account the experience of similar studies in healthy subjects [31] and patients with STBI [32]. The components of each network were verified using the AAL (Anatomical Automatic Labeling) software package.

To quantify the correlated activity of six resting fMRI networks (Fig. 1), we used such integral parameters recommended by the FSL developers as the number of voxels, their volume (cm^3) and maximum intensity. They were calculated automatically using a set of FSL console commands. Intensity refers to statistical indicators of network activity, which corresponds to the *F* value of Fisher criterion. Intensity has no units of measurement and is set automatically. *F*-value is displayed in accordance with graded color or black-and-white scale, where brightness or color corresponds to *F*-criterion value with the maximum intensity having maximal value and minimum value corresponding to the *F*-criterion value with $P=0.01$. F-values for p exceeding minimal level of significance are not displayed when anatomical and functional 3D data are combined.

The studies were performed in accordance with the principles of the Helsinki Declaration, after obtaining informed consent and approval by the ethical committee of Burdenko Scientific Research Center of the Russian Ministry of Health.

Results

Case 1. Patient M., 34 years old, suffered a road traffic accident with STBI and severe cerebral contusion, diffuse axonal injury, multiple focal hemorrhages and subarachnoid hemorrhage (MRI evidence). Following the lesion, an 8-day coma developed which transformed into PCU state.

At the time of fMRI prior to rTMS (17 days after injury), neurological examination showed severe reduction of consciousness corresponding to UWS on the CRS-R or vegetative state according to the consciousness recovery scale [33]. The motor domain showed pyramidal tetraplegia with increased muscular tone in the left arm. Limb movements assessment was 2 points, slightly better on the right side. Brainstem signs corresponding to midbrain damage were observed.

The second fMRI examination was performed 34 days after injury, on day 5 after a course (5 sessions) of therapeutic rTMS of which 1 were done in the F3 area and other 4 bilaterally in F3–F4. The consciousness was assessed as MCS+ according to the CRS-R scale or intermediate between AM and MSU, according to the consciousness recovery assessment system [33]: voluntary fixation of gaze, unsteady following of commands. In the motor domain, the movement in the left arm was 3 points, in the right arm 2 points, in the legs 3 points, the patient performed better in keeping the right leg flexed.

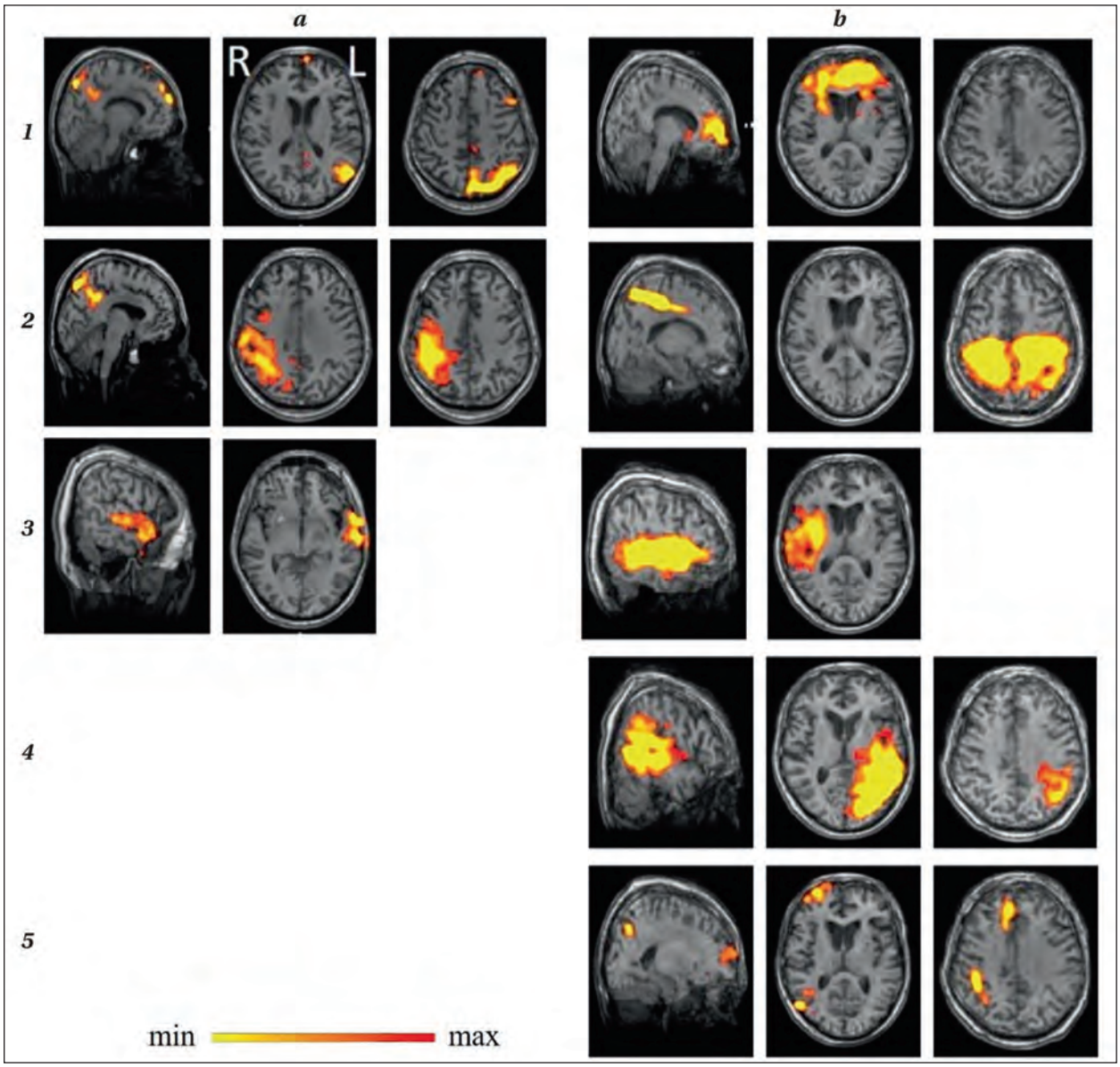

Fig. 2. Changes in RSN fMRI in patient M.
Note. *a* — prior to therapeutic rTMS; *b* — 5 days after the course of rTMS. *1* — DMN; *2* — sensorimotor; *3* — auditory; *4* — speech; *5* — frontoparietal networks.

Figure 2 shows RSN fMRI images (from those listed in the technique) of patient M. revealed before (Fig. 2, *a*) and after rTMS (Fig. 2, *b*) on identical brain slices (sagittal and horizontal).

Prior to stimulation, only 3 out of the 6 networks under study could be verified, i. e., DMN, sensorimotor, and auditory networks. Meanwhile, even the identified networks were different from normal, primarily because of their asymmetry. In the DMN (Fig. 2, *a 1*), the left hemispheric occipital component was dominant, with a pronounced reduction of the frontal component. The sensorimotor network (Fig. 2, *a 2*) was represented by activated components in the deep parts of the right hemisphere. The auditory network (Fig. 2, *a 3*) was represented by left-sided activation in the basal parts.

After stimulation, we noticed a quantitative increase in the intensity of the already existing RSNs (Table 2), with changes in their spatial organization. Thus, there was an increased bilateral activation of the anterior (frontal) component of the DMN (Fig. 2, *b 1*), as well as of the motor cortex of both hemispheres of the sensorimotor network (Fig. 2, *b 2*). We observed widespread activation of the right hemispheric component of the auditory network (Fig. 2, *b 3*). In addition, components of two RSNs that were absent before stimulation were identified: the left hemispheric posterior temporal corresponding to the speech network (Fig. 2, *b 4*), as well as the main components of the right frontoparietal network (Fig. 2, *b 5*). All of the observed changes in RSN activation due to synchronization

of hemodynamic signals relate to the network components close to the stimulation zones.

Case 2. Patient B., a 35-year-old female with a right-sided traumatic brain injury (severe contusion with intracerebral hematoma of the right frontal lobe followed by decompressive cranial trepanation in the right fronto-temporo-parietal region and hematoma removal), subsequent inflammatory process (meningoencephalitis, ventriculitis resulting in posttraumatic postinflammatory multilocular hydrocephalus followed by ventriculo-peritoneal shunting), who still remained in a long-term PCU 17 months after injury.

At the time of the examination before rTMS, the level of consciousness on the CRS-R scale was assessed as MCS-, manifested as AM (arbitrary fixation of gaze without following instructions and verbal production) [33]. Gross tetraparesis with increased muscular tone and decreased reflexes were revealed in the motor domain. MR tomograms (Fig. 3) visualized abnormalities predominantly in the right hemisphere (expansion of the right lateral ventricle and moderate lateral dislocation of the lateral ventricles to the right) with disruption of the anatomical relations of the brain structures. In the left frontal lobe, in the projection of the anterior border of the precentral gyrus, a small area of altered cerebral tissue, probably of hemorrhagic nature, was also identified.

After 9 days and 6 sessions of rTMS (5 in the F3 zone, 1 in the F4 zone), positive changes in consciousness were noted: MCS+ on the CRS-R scale, or transition to mutism with speech understanding (i.e., following selected commands) according to the consciousness recovery assessment system [33]. In the motor domain, gross tetraparesis persisted, but with a slight increase in muscular tone and reflexes. For the first time since the injury, patient B. was able to raise and lower her right arm upon request.

Fig. 3 shows the fMRI images listed in the RSN procedure of Patient B. before (Fig. 3, *a*) and after rTMS (Fig. 3, *b*) on identical brain slices (sagittal and horizontal).

Before stimulation, 6 RSNs under study were revealed including DMN, sensorimotor, executive functions, auditory, and frontoparietal, which, however, differed sharply from the normal ones. The pronounced asymmetry of all networks, with activation of components mainly in the left (structurally more intact) hemisphere, as well as their disorganization, was remarkable. The DMN was characterized by time-differentiated synchronization of the anterior (frontal) (Fig. 3, *a 1*) and posterior components, which are normally activated simultaneously. The sensorimotor network (Fig. 3, *a 2*) was represented only by the left-hand motor component, the executive function network (Fig. 3, *a 3*) by the frontal one, and the auditory network (Fig. 3, *a 5*) by the temporal one, also in the left hemisphere. The frontal-parietal network (Fig. 3, *a 4*) was activated solely superficially, in the right hemisphere, with a slight partitioning into the parietal and frontal components.

After stimulation, certain changes in resting networks were seen corresponding to positive clinical progress. All RSNs were still asymmetrically activated in the left (more morphologically intact) hemisphere. Meanwhile, greater (simultaneous) coherence of activation of the anterior and posterior components was noted in the DMN (Fig. 3, *b 1*). The left hemispheric components of the sensorimotor network (Fig. 3, *b 2*) were represented at all levels of the hemisphere, with increased intensity (Table 2), approaching normal topography. Bilateral activation of the frontal components was observed in the executive functions network (Fig. 3, *b 3*). The left frontoparietal network (Fig. 3, *b 4*) with a clear activation of both of its components was seen. While there were no significant changes in the auditory network (Fig. 3, *b 5*), the presence of fragments of the speech RSN (Fig. 3, *b 6*), undetectable prior to rTMS, was noted.

Case 3. Patient Ya., 31 years old, suffered a traumatic brain injury with severe left frontal lobe contusion, diffuse axonal damage, multiple small cortical-subcortical hemorrhagic foci, intraventricular and subarachnoid hemorrhages and subsequently developed hyporesorptive hydrocephalus according to MRI.

At the time of the examination before rTMS, 4.5 years after injury, the patient's consciousness was assessed as MCS- according to the CRS-R scale, manifested as akinetic mutism according to the consciousness recovery assessment system [33]. In the motor domain, we detected predominantly right-sided tetraparesis with increased muscular tone in the arms; brainstem signs from the midbrain level and gross pseudobulbar syndrome. MRI scans (Fig. 4) showed enlargement of the lateral ventricles, more pronounced in the anterior horns, amid brain atrophy prevailing in the frontal lobes.

Seventeen days after the course of rTMS (10 sessions in the F3–F4 areas), the patient's condition did not change, and he was still rated as MCS on the CRS-R scale. Though when asssessed according to the alternative classification [33], the patient became emotionally reactive while still having akinetic mutism. Pyramidal tetraplegia persisted, but without a significant increase in muscle tone; there were brainstem signs corresponding to the midbrain lesion, as well as a gross pseudobulbar syndrome.

Fig. 4 depicts the studied RSN fMRI of patient Ya. before (Fig. 4, *a*) and after rTMS (Fig. 4, *b*).

Before stimulation, components of 5 of the 6 resting fMRI networks under study were detected

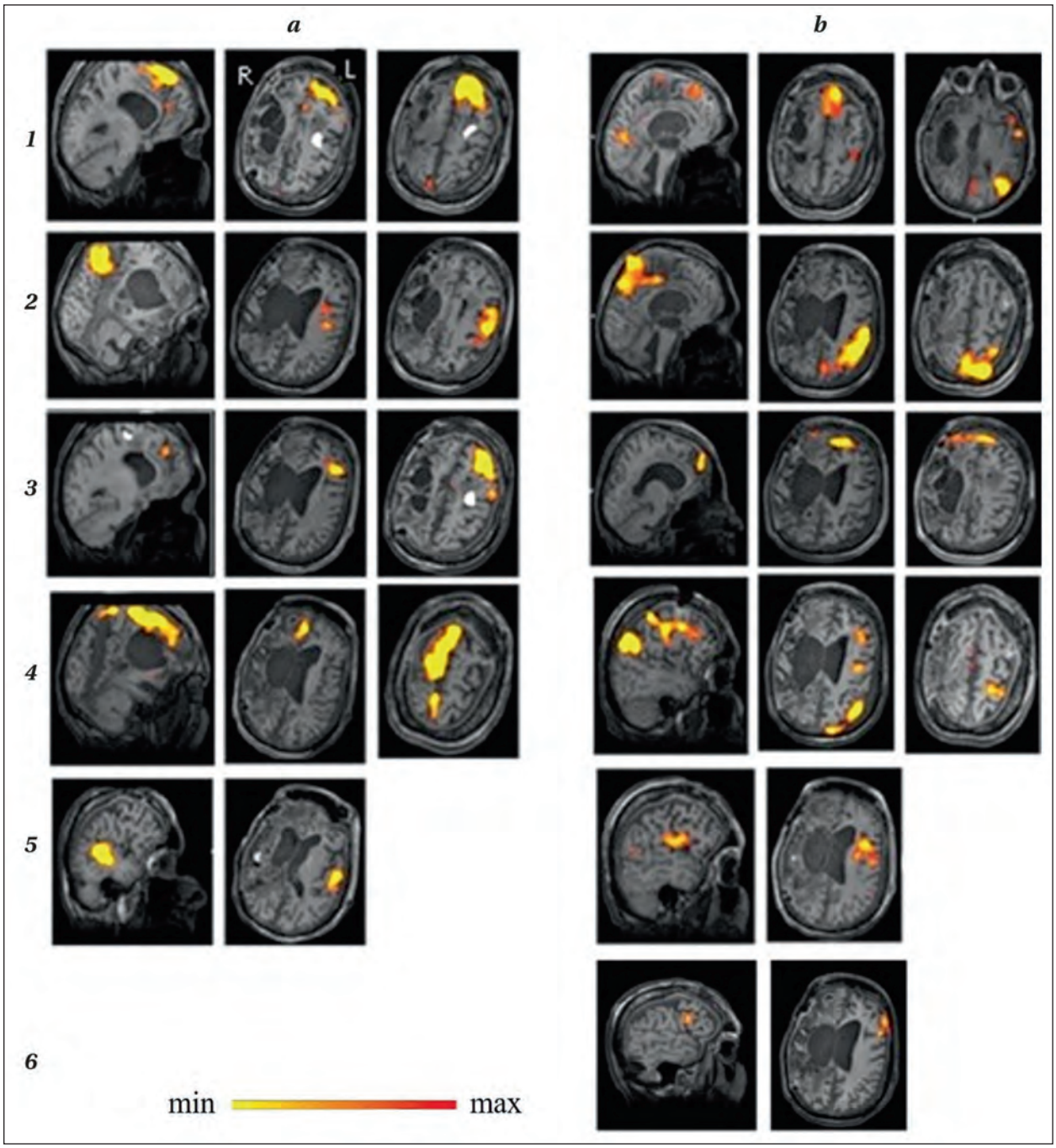

Fig. 3. Changes in RSN fMRI of patient B.
Note. *a* — before the therapeutic rTMS course; *b* — after stimulation. The RSN fMRI: *1* — DMN; *2* — sensorimotor; *3* — executive functions; *4* — frontoparietal; *5* — auditory; *6* — speech, frontal component.

such as DMN, sensorimotor, executive functions, fronto-parietal, temporal, and even speech. Similarly to Patient B., the network activation was fragmentary and predominantly unilateral, asymmetrical in nature. We detected a frontal component of the DMN, activation of the left motor cortex sensorimotor network, the left frontal cortex executive functions network, the parietal component of the right fronto-parietal network, and the left parietal component of the speech network (Fig. 4, *a 1–5*, respectively).

After stimulation, RSNs were still represented only by separate components, predominantly in the left hemisphere. The topography of the sensorimotor and executive networks did not change (Fig. 4 *b 2, 3*, respectively). The parieto-occipital component of the DMN (Fig. 4, *b 1*), the parietal component of the frontal-parietal network (Fig. 4, *b 4*), and the frontal component of the speech network (Fig. 4, *b 5*) were activated, all in the left hemisphere. Noteworthy is the appearance of a near-normal configuration

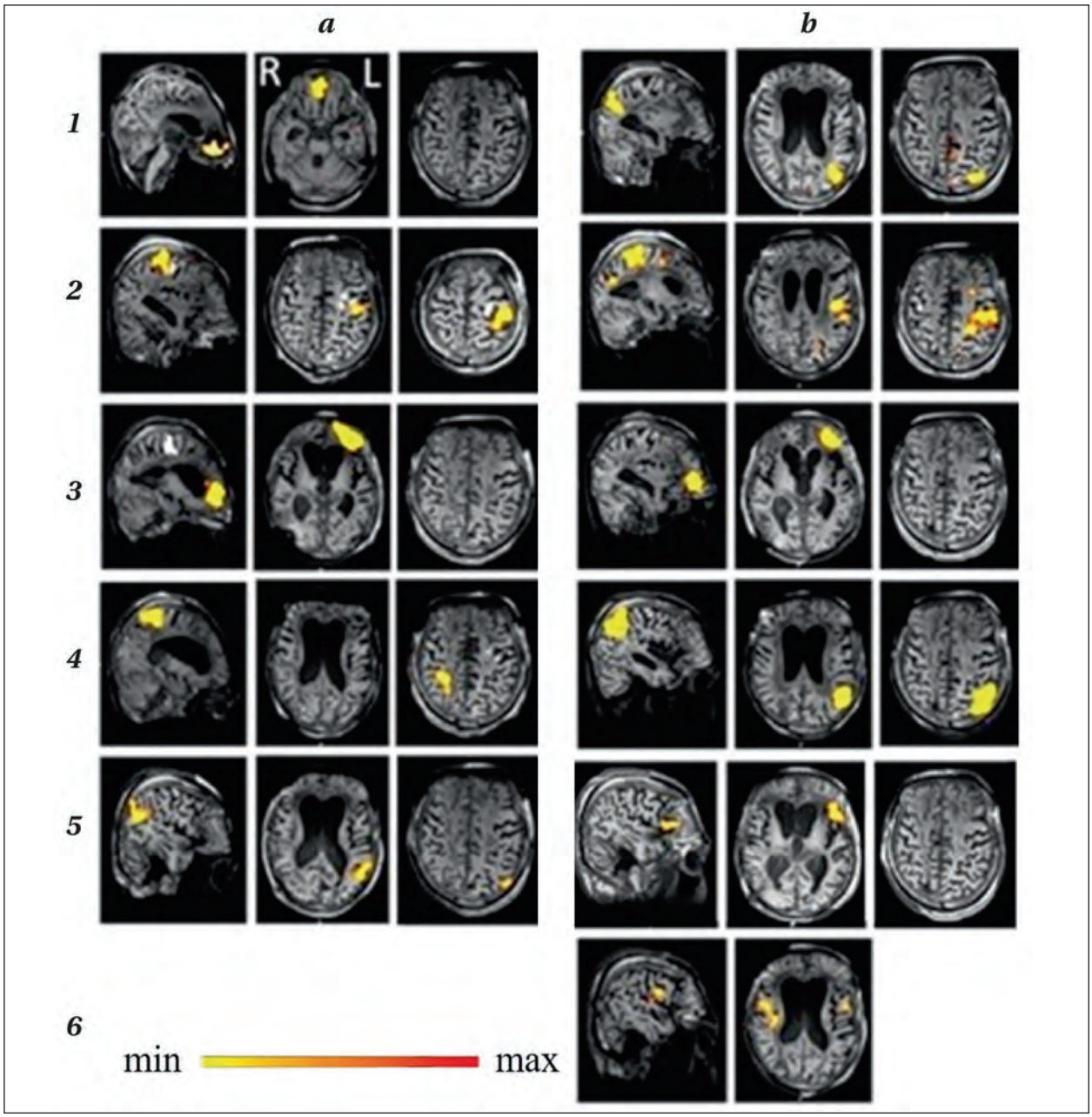

Fig. 4. Changes in RSN fMRI of patient Ya.
Note. *a* — before the rTMS course; *b* — after stimulation. RSN fMRI: *1* — DMN; *2* — sensorimotor; *3* — executive functions; *4* — fronto-parietal; *5* — speech; *6* — auditory.

of the auditory network with its bilateral components (Fig. 4, *b 6*). The intensity of the majority of the network signals visually manifested itself without pronounced changes, but with a trend toward a quantitative decrease after rTMS (Table 2). Only for the left frontoparietal network did this parameter increase.

Discussion

In all three patients with PCU states, resting fMRI networks significantly differed from normal ones both in the number of RSNs detected (reduced quantity) and in their spatial organization. They were asymmetric and had abnormal temporal synchronization of the activity of intra-network components. These results agree with the available literature data [32, 35]. Characteristic abnormalities also include the reduction in some network components, primarily cortical ones, while stem and basal components remain intact [36]. The lowest number of RSNs was detected in patient M., who was in a vegetative state before rTMS. In two ceses with akinetic mutism, the network abnormalities

Table 2. Maximal intensity of RSN fMRI before and after stimulation.

Patient	Network											
	DMN		Sensorimotor		Salience		Frontoparietal		Temporal		Speech	
	before	after	before	after	before	after	before	after	before	after	before	after
M.	11.3	17.8	13	19.1		10.3		12.8	9.4	14.3		14.3
B.	11.4	29.6	15.7	20.4	12	17.4	21	30.7		9.4	9.2	15.4
Ya.	22.2	18.6	15.7	14	33.3	11.2	12.5	20.7		11.3	11.3	10.8

mainly concerned spatial organization of the cortical components, which had been previously reported in the literature [37].

These topographic network abnormalities were essentially similar in patients B. and Ya. who were in the AM before rTMS, but with different stimulation efficiencies. In this regard, the results of the study, as well as our earlier evaluation of fMRI motor network connectivity [38], indicate significant diagnostic yield of resting fMRI parameters. The combination of fMRI recorded at rest and during any activity seems to be more efficient for prognostic purposes [38, 39]. In this study, rTMS served as test activation to a certain extent.

The common effects of rTMS in all patients include greater reactivity of the left hemisphere, regardless of the lateralization of the predominant brain lesion in patients with STBI (which we previously observed in healthy subjects as well [17]) and marked functional changes primarily in the components close to the stimulation areas (left frontal and anterior temporal areas) which were significant for the formation of almost all of the RSNs studied.

Positive neural network effects of rTMS, accompanying improvement of M. and B. patients to MCS+, manifested as a trend to normalization of spatial organization of RSNs significant for PCU state. This normalization included simultaneous synchronisation of all components with one network and appearance of networks not detected before stimulation (probably «sleeping» in lower levels of consciousness). Mainly it concerns bilateral activation of symmetric frontal or motor areas, as well as frontal and parietal regions of the left hemisphere. We believe that these changes are caused by restoration of some interhemispheric and extended intrahemispheric (fronto-parietal, etc.) functional connections. Significance of these functional and structural connectivities for development and regression of PCU was shown in several multidisciplinary studies [40–43]. Quantitatively confirmed Increase in intensity of resting networks after a course of rTMS was revealed in patients with a positive clinical effect of rTMS (qualitative improvement of the status). However, the assessed RSN fMRI «total volume» parameter did not change in parallel with the clinical evolution.

A qualitative improvement in the consciousness of patient M., who had a short-term course of rTMS early after traumatic brain injury while receiving continuous drug therapy, was remarkable. This result indicates the promising use of rTMS to activate the natural processes of neuroplasticity. Additional placebo-controlled studies are required to clarify the effect of rTMS in accelerating recovery in the early posttraumatic periods.

Less prominent consciousness recovery in patient Ya. (within the MCS-level) was manifested by activation of additional neural network components of the RSN without normalization of the spatial organization of each network (i. e., without signs of restoration of extended intrahemispheric connectivity), but with activation of symmetrical components of the temporal network, reflecting probable restoration of individual interhemispheric connections.

The feasibility of functional cerebral interactions development is largely determined by the morphology of the STBI, i. e., the conduction pathways and brain substance [44], which is confirmed by MRI data from the above patients. In this regard, the timing after injury is crucial. The striking clinical and neural network positive result of rTMS was shown in patient M. early after the STBI. Stimulation of patient Ya. 4.5 years after trauma has been the least effective.

Conclusion

Therapeutic rTMS of the frontal hemispheres can activate resting neural networks or their individual components with a tendency to normalize the RSN topology.

Our observations showed the promise of rTMS as a therapeutic intervention, as well as the feasibility of using RSN fMRI for diagnostic purposes in PCU following STBI.

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Comorbidities Coma Scale (CoCoS): Linguistic and Cultural Adaptation of the Russian-Language Version

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Summary

Identification of complications and control of comorbidities are essential in monitoring the patients with chronic disorders of consciousness and predicting their outcomes. The researchers of the Department of Biotechnological and Applied Clinical Sciences of the University of L'Aquila (Italy) developed the Comorbidities Coma Scale (CoCoS) for a comprehensive assessment of such patients. Lack of an officially validated version of the scale hampers its use in Russia, while using versions which have not been completely validated prevents clinicians from obtaining reliable results when examining patients with chronic disorders of consciousness.

Aim. To develop the official Russian language version of the Comorbidities Coma Scale, considering various linguistic and cultural parameters, as a part of the 1st stage of the validation study.

Material and methods. The first stage of validation was completed: direct and reverse translation of the scale was performed by two independent medical translators. The translated version was assessed by an expert board including an expert translator, neurologists, and critical care specialists. Pilot test and two meetings of the expert board, before and after testing, were arranged to assess the results and approve the final Russian version of the scale.

Results. During the first meeting of the expert board, corrections were made in the Russian language version of the scale in terms of language and cultural adaptation. Pilot testing was carried out based on the inclusion and exclusion criteria. The researchers had no difficulties in understanding and interpreting the instructions for the scale. The second meeting of the expert board was held thereupon, and the final version of the Russian language version of the scale was adopted, which is available on the website of the Center for Validation of Health Status Questionnaires and Scales of the Research Center of Neurology.

Conclusion. The first stage of validation, i. e., linguistic and cultural adaptation, was carried out at the Research Center of Neurology (Moscow, Russia). For the first time, the Russian version of the scale for assessing comorbidities in patients with chronic disorders of consciousness was presented and approved for the practical use. The future publications will address the psychometric results of the scale such as sensitivity, validity, reliability.

Keywords: Comorbidities Coma Scale; CoCoS; chronic disorders of consciousness; vegetative state; unresponsive wakefulness syndrome; minimally conscious state; validation

Conflict of interest. The authors declare no conflict of interest.

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Шкала оценки сопутствующих заболеваний у ареактивных пациентов (CoCoS): лингвокультурная адаптация русскоязычной версии (сообщение)

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Резюме

Выявление осложнений и контроль над течением сопутствующих заболеваний является важнейшим этапом в отслеживании динамики и прогнозе исходов у пациентов с хроническими нарушениями сознания. Для проведения оценки состояния у данной категории пациентов сотрудниками департамента биотехнологических и прикладных клинических наук университета L'Aquila (Италия) была разработана шкала — Comorbidities Coma Scale (CoCoS). Отсутствие официально валидированной версии данной шкалы затрудняет ее применение в России, а использование версий, не прошедших все необходимые этапы валидации, препятствует получению достоверных результатов при обследовании пациентов с хроническими нарушениями сознания.

Цель. Разработка официальной русскоязычной версии Шкалы оценки сопутствующих заболеваний у ареактивных пациентов с учетом языковых и культурных особенностей ее пользователей в рамках проведения 1-го этапа валидационного исследования.

Материал и методы. Письменное разрешение на адаптацию шкалы CoCoS было получено сотрудниками группы валидации международных шкал и опросников Научного центра неврологии (ФГБНУ НЦН, г. Москва, Россия) у разработчика оригинальной версии Francesca Pistoia. Провели первый этап валидации: выполнен прямой и обратный перевод шкалы двумя независимыми медицинскими переводчиками. Произведена оценка разработанной версии экспертной комиссией с участием переводчика-эксперта, неврологов и анестезиологов-реаниматологов. Провели пилотное тестирование на 15 пациентах с диагнозом хронического нарушения сознания и два заседания экспертной комиссии до и после тестирования для оценки результатов и утверждения окончательной русскоязычной версии шкалы.

Результаты. В ходе первого заседания экспертной комиссии внесли поправки в русскоязычную версию шкалы в рамках языковой и культурной адаптации: были изменены единицы измерения лабораторных показателей с мг/дл на ммоль/л в 7-м и 14-м пунктах (оценка гликемии и концентрации креатинина, соответственно). Изменен термин «надаортальные сосуды» на «брахиоцефальные артерии» в 10-м пункте, сопоставлены предложенные варианты повреждения мягких тканей со стадиями развития пролежней согласно NPUAP — EPUAP [18] в 21-м пункте, добавлен параметр индекс массы тела (ИМТ) для оценки выраженности недостаточности питания. В ходе пилотного тестирования с учетом критериев включения и исключения сложностей при понимании и интерпретации инструкций шкалы у исследователей не возникло. По итогам состоялось второе заседание экспертной комиссии, на котором приняли окончательный вариант русскоязычной версии шкалы. Он доступен для ознакомления на сайте группы валидации международных шкал и опросников ФГБНУ НЦН https://www.neurology.ru/reabilitaciya/centr-validacii-mezhdunarodnyh-shkal-i-oprosnikov, а также по QR-коду:

Заключение. На базе ФГБНУ НЦН выполнили первый этап валидации — лингвокультурную адаптацию. Впервые представили и рекомендовали к использованию русскоязычную версию Шкалы оценки сопутствующих заболеваний у ареактивных пациентов. В последующих публикациях будут представлены результаты оценки психометрических свойств (чувствительность, валидность, надежность) русскоязычной версии данной шкалы.

Ключевые слова: Comorbidities Coma Scale; CoCoS; коморбидность; сопутствующие заболевания; хронические нарушения сознания; вегетативное состояние; синдром ареактивного бодрствования; состояние минимального сознания; валидация

Конфликт интересов. Авторы заявляют об отсутствии конфликта интересов.

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Введение

Хронические нарушения сознания (ХНС) — это состояния, чаще развивающиеся после комы и характеризующиеся восстановлением бодрствования при частичном или полном отсутствии признаков осознанного поведения (осознания окружающей действительности и собственной личности), длящиеся не менее 28 дней от мо-

мента возникновения нарушения сознания [1]. Основные синдромы ХНС представлены вегетативным состоянием или синдромом ареактивного бодрствования (ВС/САБ) [2], и состоянием минимального сознания (СМС) «минус» или «плюс» [3].

Определить прогноз у пациентов с ХНС — довольно сложная задача в связи с трудностью проведения долгосрочных исследований у этой категории пациентов и оценки влияния большого количества факторов на течение заболевания. Тем не менее, наличие осложнений и/или сопутствующих заболеваний является одним из общепризнанных факторов восстановления сознательной деятельности, функциональной независимости и, в целом, определяющих прогноз в отношении выживания, что подтверждает его клиническую значимость.

В настоящее время существует несколько международных индексов и шкал для оценки коморбидности: система Cumulative Illness Rating Scale (CIRS) [4], индекс коморбидности Charlson [5], индекс Kaplan–Feinstein [6], индекс сосуществующих болезней (ICED) [7], гериатрический индекс коморбидности [8], индекс функциональной коморбидности (FTI) [9], индекс Total Illness Burden Index (TIBI) [10]. Ограничение в использовании данных клинических инструментов состоит в том, что они разработаны для пациентов с другими диагнозами, спектр сопутствующих заболеваний которых отличается от встречающихся у пациентов с ХНС. Используя представленные выше шкалы и индексы, невозможно надежно оценить у пациентов с ХНС такие состояния, как депрессия, потеря слуха, предшествующие когнитивные нарушения из-за отсутствия осознанного поведения.

Выявление осложнений и контроль над течением сопутствующих заболеваний является важнейшим этапом в отслеживании динамики и прогнозе исходов данной категории пациентов. Было показано, что декомпенсация соматической патологии значительно влияет на выживание, восстановление сознания и функциональной независимости [11, 12]. С этой целью в 2019 г. сотрудниками департамента биотехнологических и прикладных клинических наук университета L'Aquila (Италия) была разработана Шкала оценки сопутствующих заболеваний у ареактивных пациентов (*англ.* Comorbidities Coma Scale — CoCoS) [13].

Шкала включает 24 категории, каждая из которых основана на наличии или отсутствии конкретных сопутствующих заболеваний и степени их тяжести. Категории патологических состояний включают: инфекции дыхательных и мочевыводящих путей, неинфекционные заболевания дыхательных путей, заболевания сердца ишемической или неишемической этиологии, нарушения сердечного ритма, артериальную гипертензию, сахарный диабет, пароксизмальную симпатическую гиперактивность, заболевания периферических и брахиоцефальных артерий, периферических вен, заболевания гепатобилиарной системы, желудочно-кишечного тракта, почек, судорожные приступы, гидроцефалию, наличие переломов, устройств для поддержания жизнедеятельности, пролежней, анемию, заболевания суставов, злокачественные опухоли, недостаточность питания, предшествующую инвалидизацию. Степень тяжести каждой из категории оценивается с помощью предложенных вариантов. Например, тяжесть респираторных инфекций оценивается на основании отсутствия/наличия симптомов, потребности в лечении и реакции на лечение, а тяжесть анемии определяется на основании содержания гемоглобина [13].

Применение шкалы требует изучения истории заболевания, оценку соматического и неврологического статуса пациента, данных инструментальных и лабораторных исследований. В случае если данных для анализа недостаточно, необходимо отметить пункт «оценка невозможна», который предлагается после каждой категории заболевания. Стоит отметить, что при отметке «оценка невозможна» по двум и более показателям, во избежание неправильного определения степени тяжести сопутствующих заболеваний, сумма баллов не учитывается. По результатам проведенной оценки рассчитывается общая сумма баллов, которая показывает отсутствие (0 баллов) или наличие сопутствующих заболеваний легкой (1–24 балла), умеренной (25–48 баллов) или тяжелой (49–72 балла) степени.

Разработчики шкалы провели валидационное исследование оригинальной версии на выборке из 162 пациентов с нарушениями сознания (кома, ВС/САБ, СМС) травматической и нетравматической этиологии. Была получена высокая согласованность оценок врачей-экспертов при повторном тестировании, которая показала, что шкала может быть использована для выявления сопутствующих заболеваний в повседневной клинической практике, а также для изучения их влияния на прогноз [13]. Стоит отметить, что на настоящий момент по результатам поиска по базе данных PubMed и Google Scholar оригинальное исследование 2019 г. является единственным, перевод на другие языки и адаптация шкалы для неанглоязычной популяции не проводились.

Для получения надежных результатов при обследовании пациентов с нарушениями сознания, используя различные зарубежные шкалы, необходимо валидировать вышеупомянутые

диагностические инструменты для русскоязычных пациентов, учитывая языковые и культурные особенности [14–16].

Отсутствие процедуры валидации препятствует возможности сопоставления результатов с данными, полученными другими исследователями. Принимая во внимание, что шкала CoCoS является надежным и необходимым инструментом для оценки тяжести сопутствующих заболеваний у пациентов с ХНС, разработка официальной русскоязычной версии шкалы, прошедшей этап лингвокультурной адаптации с последующей оценкой психометрических свойств, будет способствовать повышению доступности шкалы для использования в русскоговорящих странах, а также повысит достоверность полученных результатов.

Цель работы — разработка официальной русскоязычной версии шкалы CoCoS и проведении пилотного тестирования в рамках первого этапа валидационного исследования данного клинического инструмента.

Материал и методы

Письменное разрешение на адаптацию шкалы CoCoS было получено сотрудниками группы валидации международных шкал и опросников Научного центра неврологии (ФГБНУ НЦН, г. Москва, Россия) у разработчика оригинальной версии Francesca. Pistoia. Лингвокультурную адаптацию проводили согласно общепринятым рекомендациям [17]. Первый этап включал в себя прямой перевод шкалы с английского на русский язык двумя независимыми медицинскими переводчиками. Следующий этап включал формирование комбинированной версии из полученных переводов и обратный перевод на английский язык носителями языка с медицинским образованием. Проведена оценка разработанной версии экспертной комиссией под председательством переводчика-эксперта, не принимавшего ранее участия в работе над переводом. В состав комиссии также вошли следующие специалисты: неврологи, работающие в отделении реанимации более 5 лет, анестезиологи-реаниматологи, медицинские переводчики.

Провели пилотное тестирование на 15 пациентах с диагнозом хронического нарушения сознания, проходивших лечение в отделении анестезиологии-реанимации с палатами реанимации и интенсивной терапии ФГБНУ «Научный центр неврологии».

Критериями включения в исследование послужило наличие острого (кома) или хронического нарушения сознания у пациента старше 18 лет при наличии письменного согласия представителя пациента. Среди критериев исключения были: длительность хронического нарушения сознания более 1 года, а также наличие жизнеугрожающих состояний.

На повторном заседании экспертной комиссии проведена оценка результатов пилотного тестирования и утверждена окончательная русскоязычная версия шкалы.

Результаты и обсуждение

Согласно данным мировой литературы у пациентов с ХНС практически всегда присутствуют сопутствующие заболевания, влияющие на прогноз и исход [11–12]. Некоторые из этих заболеваний можно отнести к преморбидным состояниям (например, артериальная гипертензия, нарушения сердечного ритма), тогда как другие являются осложнениями основного заболевания (в том числе, развитие контрактур и пролежней вследствие длительной иммобилизации и не в полной мере осуществимого ухода).

Вышеперечисленные состояния вызывают декомпенсацию соматического статуса, что способно ограничить возможность проведения восстановительного лечения и уменьшить вероятность функционального восстановления.

Во время заседания экспертной комиссии основные сложности, возникшие в процессе перевода и формировании окончательной русскоязычной версии, были связаны с адаптацией первоначального смысла англоязычного текста. Обсуждали два варианта названия «Шкала оценки сопутствующих заболеваний у ареактивных пациентов» и «Шкала оценки коморбидности у ареактивных пациентов» по результатам дискуссии было принято решение отдать предпочтение первому варианту как более подходящему с медицинской и лингвистической точки зрения. Также, во время заседания комиссии было внесено несколько поправок в целях повышения информативности и доступности для понимания носителями русского языка (т. е. в рамках языковой и культурной адаптации). В частности, были изменены единицы измерения лабораторных показателей с мг/дл на ммоль/л в 7-м и 14-м пунктах (оценка гликемии и концентрации креатинина, соответственно). Изменен термин «надаортальные сосуды» на «брахиоцефальные артерии» в 10-м пункте, сопоставлены предложенные варианты повреждения мягких тканей со стадиями развития пролежней согласно NPUAP — EPUAP [18] в 21-м пункте, добавлен параметр индекс массы тела (ИМТ) для оценки выраженности недостаточности питания.

Следующим этапом стало проведение пилотного тестирования с участием 15 пациентов с длительностью ХНС, согласно критериям исключения, менее одного года. Оценка по шкале проводилась двумя исследователями на следующий день после поступления пациента и через неделю после первого обследования для оценки динамики состояния. Медиана и межквартильный интервал возраста пациентов составили 30 (23–46) лет. Из 15 пациентов с ХНС с диагнозом «ВС/САБ» составило 7, «СМС» — 8.

Сложностей при понимании и интерпретации инструкций у исследователей не возникло.

По результатам пилотного исследования проведено повторное заседание экспертной комиссии и утвержден окончательный вариант русскоязычной версии шкалы.

С русскоязычной версией Шкалы оценки сопутствующих заболеваний у ареактивных пациентов можно ознакомиться в приложении, а также на сайте группы валидации международных шкал и опросников ФГБНУ НЦН https://www.neurology.ru/reabilitaciya/centr-validacii-mezhdunarodnyh-shkal-i-oprosnikov и по QR-коду:

Заключение

Шкала оценки сопутствующих заболеваний у ареактивных пациентов является необходимым клиническим инструментом для объективной оценки тяжести соматической патологии у пациентов с хроническими нарушениями сознания. Провели первый этап валидации — лингвокультурная адаптация шкалы для русскоязычного населения. На момент публикации продолжается набор пациентов для оценки психометрических свойств (чувствительность, валидность, надежность) русскоязычной версии данной шкалы.

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Приложение

Шкала оценки сопутствующих заболеваний у ареактивных пациентов(CoCoS)

1. ИНФЕКЦИИ ДЫХАТЕЛЬНЫХ ПУТЕЙ

0. Отсутствуют
1. Симптомы, подтвержденные или не подтвержденные инструментальными/лабораторными данными, требующие или не требующие лечения
2. Рецидивирующие или резистентные к терапии инфекции
3. Септический шок

☐ Оценка невозможна
☐ Ранее существовавшее заболевание
☐ Впервые диагностированное заболевание
☐ Как впервые диагностированное, так и ранее существовавшее заболевание

2. ИНФЕКЦИИ МОЧЕВЫВОДЯЩИХ ПУТЕЙ

0. Отсутствуют
1. Наличие изменений в анализе мочи (лейкоцитурия) при отсутствии симптомов
2. Наличие изменений в анализе мочи (лейкоцитурия), а также симптомов(лихорадка, гематурия, изменение физических свойств мочи), требующих лечения, либо наличие резистентных к терапии инфекций
3. Септический шок

☐ Оценка невозможна
☐ Ранее существовавшее заболевание
☐ Впервые диагностированное заболевание
☐ Как впервые диагностированное, так и ранее существовавшее заболевание

3. НЕИНФЕКЦИОННЫЕ ЗАБОЛЕВАНИЯ ДЫХАТЕЛЬНЫХ ПУТЕЙ

0. Отсутствуют
1. Бессимптомное заболевание даже при наличии в анамнезе обструктивных или рестриктивных болезней легких
2. Обструктивные или рестриктивные болезни легких, требующие лечения или хорошо контролируемые с помощью проводимого лечения
3. Обструктивные или рестриктивные болезни легких с рецидивирующей или не поддающейся лечению дыхательной недостаточностью

☐ Оценка невозможна
☐ Ранее существовавшее заболевание
☐ Впервые диагностированное заболевание
☐ Как впервые диагностированное, так и ранее существовавшее заболевание

4. ОРГАНИЧЕСКОЕ ЗАБОЛЕВАНИЕ СЕРДЦА (ишемической или неишемической этиологии)

0. Отсутствует
1. Бессимптомный эпизод или наличие эпизода в анамнезе без остаточных симптомов
2. Одышка (частота дыхания > 20 в минуту и/или наличие симптомов, позволяющих заподозрить стенокардию, периферические отеки легкой или умеренной степени при наличии инструментальных признаков заболевания сердца (по данным ЭКГ, УЗИ сердца)), хорошо контролируемая проводимым лечением
3. Кардиогенный шок (систолическое АД < 90 мм рт. ст., признаки гипоперфузии, включая олигурию и снижение сердечного выброса, при наличии или отсутствии симптомов отека легких)

☐ Оценка невозможна
☐ Ранее существовавшее заболевание
☐ Впервые диагностированное заболевание
☐ Как впервые диагностированное, так и ранее существовавшее заболевание

5. НАРУШЕНИЯ СЕРДЕЧНОГО РИТМА ПРИ ОТСУТСТВИИ ОРГАНИЧЕСКОГО ЗАБОЛЕВАНИЯ СЕРДЦА

0. Отсутствуют
1. Нарушения бессимптомные, присутствуют только изменения на ЭКГ

2. Присутствуют симптомы (например, эпизоды стойкой тахикардии, брадикардии), которые купируются с помощью лечения
3. Заболевание не поддается контролю при помощи терапии

☐ Оценка невозможна
☐ Ранее существовавшее заболевание
☐ Впервые диагностированное заболевание
☐ Как впервые диагностированное, так и ранее существовавшее заболевание

6. АРТЕРИАЛЬНАЯ ГИПЕРТЕНЗИЯ

0. Отсутствует
1. Артериальная гипертензия в анамнезе или пограничная артериальная гипертензия, не требующая лечения
2. Артериальная гипертензия, которая хорошо контролируется с помощью лечения
3. Артериальная гипертензия, которая не контролируется с помощью лечения

☐ Оценка невозможна
☐ Ранее существовавшее заболевание
☐ Впервые диагностированное заболевание
☐ Как впервые диагностированное, так и ранее существовавшее заболевание

7. САХАРНЫЙ ДИАБЕТ

0. Отсутствует
1. Нарушение толерантности к глюкозе (гликемия 5,6–6,9 ммоль/л)
2. Сахарный диабет (гликемия ⩾ 7,0 ммоль/л), который контролируется с помощью проводимого лечения
3. Неконтролируемый сахарный диабет или наличие хронических осложнений

☐ Оценка невозможна
☐ Ранее существовавшее заболевание
☐ Впервые диагностированное заболевание
☐ Как впервые диагностированное, так и ранее существовавшее заболевание

8. ПАРОКСИЗМАЛЬНАЯ СИМПАТИЧЕСКАЯ ГИПЕРАКТИВНОСТЬ

(тахикардия (ЧСС >120 ударов/мин), тахипноэ (частота дыхания > 30 в 1 минуту), артериальная гипертензия (систолическое артериальное давление > 160 мм рт. ст.), гипертермия или гипотермия, обильное потоотделение, децеребрационная или декортикационная поза, повышение мышечного тонуса, пилоэрекция, покраснение лица)

0. Отсутствуют
1. Наличие одного из симптомов
2. Наличие двух или более симптомов, требующих проведения лечения и контроля
3. Состояние не контролируется с помощью лечения

☐ Оценка невозможна
☐ Ранее существовавшее заболевание
☐ Впервые диагностированное заболевание
☐ Как впервые диагностированное, так и ранее существовавшее заболевание

9. ЗАБОЛЕВАНИЕ ПЕРИФЕРИЧЕСКИХ АРТЕРИЙ

0. Отсутствует
1. Ранее существовавшее заболевание периферических артерий, подтвержденное данными УЗИ или наличием в анамнезе шунтирующей операции
2. Трофические изменения кожи или инструментальные данные, свидетельствующие о наличии аневризмы брюшной аорты
3. Поверхностный некроз, требующий проведения ампутации

☐ Оценка невозможна
☐ Ранее существовавшее заболевание
☐ Впервые диагностированное заболевание
☐ Как впервые диагностированное, так и ранее существовавшее заболевание

10. ЗАБОЛЕВАНИЕ БРАХИОЦЕФАЛЬНЫХ АРТЕРИЙ

0. Отсутствует
1. Стеноз одной артерии, отходящей от дуги аорты < 70%
2. Стеноз обеих артерий, отходящих от дуги аорты < 70% или стеноз одной артерии, отходящей от дуги аорты > 70%, или наличие в анамнезе каротидной эндартерэктомии с одной стороны

3. Стеноз обеих артерий, отходящих от дуги аорты >70% или наличие в анамнезе каротидной эндартерэктомии с двух сторон

☐ Оценка невозможна
☐ Ранее существовавшее заболевание
☐ Впервые диагностированное заболевание
☐ Как впервые диагностированное, так и ранее существовавшее заболевание

11. ЗАБОЛЕВАНИЕ ПЕРИФЕРИЧЕСКИХ ВЕН

0. Отсутствует
1. Неосложненная варикозная болезнь вен или наличие в анамнезе тромбофлебита без остаточных явлений
2. Недостаточность глубоких вен нижних конечностей с периферическими отеками легкой или умеренной степени или с единичным эпизодом тромбоэмболии легочной артерии
3. Посттромбофлебитический синдром с тяжелыми отеками или рецидивирующими эпизодами тромбоэмболии легочной артерии и/или трофическими язвами

☐ Оценка невозможна
☐ Ранее существовавшее заболевание
☐ Впервые диагностированное заболевание
☐ Как впервые диагностированное, так и ранее существовавшее заболевание

12. ЗАБОЛЕВАНИЯ ГЕПАТОБИЛИАРНОЙ СИСТЕМЫ

0. Отсутствуют
1. Бессимптомное заболевание или перенесенный вирусный гепатит В или С, наличие в анамнезе бессимптомной желчнокаменной болезни, холецистэктомии
2. Недавно перенесенный гепатит (< 1 года), неактивный хронический гепатит
3. Цирроз печени класса В/С по классификации Чайлда–Пью

☐ Оценка невозможна
☐ Ранее существовавшее заболевание
☐ Впервые диагностированное заболевание
☐ Как впервые диагностированное, так и ранее существовавшее заболевание

13. ЗАБОЛЕВАНИЯ ЖЕЛУДОЧНО-КИШЕЧНОГО ТРАКТА

0. Отсутствуют
1. Бессимптомное заболевание с возможным наличием в анамнезе хирургического или медикаментозного лечения
2. Гастроэзофагеальная рефлюксная болезнь, гастрит с клинически выраженными симптомами, обострение язвенной болезни, дивертикулит, которые хорошо контролируются с помощью проводимого лечения
3. Осложнения перечисленных выше заболеваний (обструкция, перфорация, кровотечение)

☐ Оценка невозможна
☐ Ранее существовавшее заболевание
☐ Впервые диагностированное заболевание
☐ Как впервые диагностированное, так и ранее существовавшее заболевание

14. ЗАБОЛЕВАНИЯ ПОЧЕК

0. Отсутствуют
1. Бессимптомное заболевание или наличие в анамнезе заболевания почек или хирургического вмешательства по поводу нефролитиаза (креатинин < 132,6 мкмоль/л)
2. Неосложненное заболевание почек (креатинин 132,6–221,1 мкмоль/л) или нефролитиаз
3. Осложненное заболевание почек (креатинин > 221,1 мкмоль/л), требующее проведения диализа или трансплантации почки

☐ Оценка невозможна
☐ Ранее существовавшее заболевание
☐ Впервые диагностированное заболевание
☐ Как впервые диагностированное, так и ранее существовавшее заболевание

15. СУДОРОЖНЫЕ ПРИСТУПЫ

0. Отсутствуют
1. Единичные судорожные приступы (например, при повышении температуры тела или метаболических нарушениях), не требующие проведения противоэпилептической терапии
2. Рецидивирующие судорожные приступы, требующие проведения противоэпилептической терапии
3. Однократное или повторное развитие эпилептического статуса

- ☐ Оценка невозможна
- ☐ Ранее существовавшее заболевание
- ☐ Впервые диагностированное заболевание
- ☐ Как впервые диагностированное, так и ранее существовавшее заболевание

16. ГИДРОЦЕФАЛИЯ

0. Отсутствует
1. Нормотензивная гидроцефалия
2. Гидроцефалия,требующая проведения вентрикулоперитонеального шунтирования и/или декомпрессионной краниоэктомии в остром периоде
3. Гидроцефалия, требующая проведения экстренной декомпрессионной краниотомии после госпитализации в реабилитационный центр

- ☐ Оценка невозможна
- ☐ Ранее существовавшее заболевание
- ☐ Впервые диагностированное заболевание
- ☐ Как впервые диагностированное, так и ранее существовавшее заболевание

17. ПЕРЕЛОМЫ

0. Отсутствуют
1. Закрытый перелом без смещения
2. Открытый перелом или закрытый перелом со смещением
3. Открытый перелом со смещением

- ☐ Оценка невозможна
- ☐ Ранее существовавшее заболевание
- ☐ Впервые диагностированное заболевание
- ☐ Как впервые диагностированное, так и ранее существовавшее заболевание

18. НАЛИЧИЕ УСТРОЙСТВ ДЛЯ ПОДДЕРЖАНИЯ ЖИЗНЕДЕЯТЕЛЬНОСТИ

(трахеостомическая трубка, назогастральный зонд,гастростома, мочевой катетер, эпицистостома, центральный венозный катетер)

0. Отсутствуют
1. Только 1 устройство
2. Два устройства
3. Больше двух устройств

- ☐ Оценка невозможна
- ☐ Ранее существовавшее заболевание
- ☐ Впервые диагностированное заболевание
- ☐ Как впервые диагностированное, так и ранее существовавшее заболевание

19. АНЕМИЯ

0. Отсутствует
1. Анемия легкой степени (Hb 120–100 г/л)
2. Анемия средней или тяжелой степени (Hb 60–99 г/л)
3. Анемия тяжелой степени (Hb < 60 г/л)

- ☐ Оценка невозможна
- ☐ Ранее существовавшее заболевание
- ☐ Впервые диагностированное заболевание
- ☐ Как впервые диагностированное, так и ранее существовавшее заболевание

20. ЗАБОЛЕВАНИЯ СУСТАВОВ

0. Отсутствуют
1. Бессимптомное заболевание или спорадически возникающая боль; рентгенологические признаки дегенеративного или воспалительного заболевания
2. Персистирующая боль, ограничение объема движений легкой или умеренной степени, боль купируется с помощью проводимого лечения, наличие в анамнезе протезирования тазобедренного или коленного сустава
3. Персистирующая боль с ограничением объема движений тяжелой степени, выраженная деформация сустава, наличие гетеротопической оссификации и остеом.

- ☐ Оценка невозможна
- ☐ Ранее существовавшее заболевание
- ☐ Впервые диагностированное заболевание
- ☐ Как впервые диагностированное, так и ранее существовавшее заболевание

21. ПРОЛЕЖНИ

0. Отсутствуют; возможно покраснение интактной кожи, не исчезающее при надавливании, локализующееся, как правило, над костным выступом (1 степень)
1. Дефект кожи не на полную ее толщину в виде неглубокой открытой раны с розовым дном без некротического отделяемого (2 степень)
2. Поражение тканей на всю глубину. Может быть видна подкожная клетчатка, но сухожилия, мышцы, кость не обнажены. Может присутствовать некротическое отделяемое, однако оно не препятствует оценке глубины дефекта ткани (3 степень)
3. Поражение тканей на всю глубину с обнажением кости, сухожилия или мышцы. На некоторых участках дна раны могут присутствовать некротическое отделяемое или струп (4 степень)

☐ Оценка невозможна
☐ Ранее существовавшее заболевание
☐ Впервые диагностированное заболевание
☐ Как впервые диагностированное, так и ранее существовавшее заболевание

22. ЗЛОКАЧЕСТВЕННЫЕ ОПУХОЛИ

0. Отсутствуют
1. Диагноз установлен и лечение проведено > 5 лет назад
2. Диагноз установлен и лечение проведено < 5 лет назад
3. Терминальная стадия злокачественной опухоли

☐ Оценка невозможна
☐ Ранее существовавшее заболевание
☐ Впервые диагностированное заболевание
☐ Как впервые диагностированное, так и ранее существовавшее заболевание

23. НЕДОСТАТОЧНОСТЬ ПИТАНИЯ

0. Отсутствует
1. Легкое нарушение, определяется только по физическим параметрам
2. Умеренное нарушение, определяется по физическим параметрам и результатам лабораторных исследований (альбумин 25–34 г/л)
3. Тяжелое нарушение, определяется по физическим параметрам и результатам лабораторных исследований (альбумин < 25 г/л)

☐ Оценка невозможна
☐ Ранее существовавшее заболевание
☐ Впервые диагностированное заболевание
☐ Как впервые диагностированное, так и ранее существовавшее заболевание

24. ПРЕДШЕСТВУЮЩАЯ ИНВАЛИДИЗАЦИЯ

(в результате заболевания/травмы)

0. Предшествующая инвалидизация отсутствует
1. Предшествующая инвалидизация легкой степени со снижением независимости в повседневной жизни, посторонняя помощь в выполнении повседневных дел не требуется
2. Предшествующая инвалидизация умеренной степени, требуется посторонняя помощь в выполнении повседневных дел
3. Предшествующая инвалидизация тяжелой степени (пациент прикован к постели, имеются нарушения функции тазовых органов, полностью зависит от помощи окружающих)

☐ Оценка невозможна

Вариант ответа «Оценка невозможна»:
Если в медицинской документации пациента недостаточно данных для оценки какого-либо параметра, необходимо выбрать вариант ответа «Оценка невозможна» и объяснить причину этого в специально отведенном поле (ниже):
……
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……
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Литература

Pistoia F., Carolei A., Bodien Y.G., Greenfield S., Kaplan S., Sacco S., Pistarini C., Casalena A., De Tanti A., Cazzulani B., Bellaviti G., Sarà M., Giacino J. Comorbidities Coma Scale (CoCoS): Psychometric Properties and Clinical Usefulness in Patients With Disorders of Consciousness. *J Frontiers in Neurology,* 2020. 10: 1042. DOI: 10.3389/fneur.2019.01042

https://doi.org/10.15360/1813-9779-2022-2-65-75
For Practitioner

Форма для записи

Пациент: Диагноз: Этиология:

Дата приобретенного тяжелого поражения головного мозга: Дата проведения оценки:

	ЕЖЕМЕСЯЧНАЯ ОЦЕНКА (от 0 до 3 баллов, как указано в Шкале)	До приобретенного тяжелого поражения головного мозга	При поступлении	1	2	3	4	5	6	7	8	9	10	11	12
1	ИНФЕКЦИИ ДЫХАТЕЛЬНЫХ ПУТЕЙ														
2	ИНФЕКЦИИ МОЧЕВЫВОДЯЩИХ ПУТЕЙ														
3	НЕИНФЕКЦИОННЫЕ ЗАБОЛЕВАНИЯ ДЫХАТЕЛЬНЫХ ПУТЕЙ														
4	ОРГАНИЧЕСКОЕ ЗАБОЛЕВАНИЕ СЕРДЦА (ишемической или неишемической этиологии)														
5	НАРУШЕНИЯ СЕРДЕЧНОГО РИТМА ПРИ ОТСУТСТВИИ ОРГАНИЧЕСКОГО ЗАБОЛЕВАНИЯ СЕРДЦА														
6	АРТЕРИАЛЬНАЯ ГИПЕРТЕНЗИЯ														
7	САХАРНЫЙ ДИАБЕТ														
8	ПАРОКСИЗМАЛЬНАЯ СИМПАТИЧЕСКАЯ ГИПЕРАКТИВНОСТЬ														
9	ЗАБОЛЕВАНИЕ ПЕРИФЕРИЧЕСКИХ АРТЕРИЙ														
10	ЗАБОЛЕВАНИЕ БРАХИОЦЕФАЛЬНЫХ АРТЕРИЙ														
11	ЗАБОЛЕВАНИЕ ПЕРИФЕРИЧЕСКИХ ВЕН														
12	ЗАБОЛЕВАНИЯ ГЕПАТОБИЛИАРНОЙ СИСТЕМЫ														
13	ЗАБОЛЕВАНИЯ ЖЕЛУДОЧНО-КИШЕЧНОГО ТРАКТА														
14	ЗАБОЛЕВАНИЯ ПОЧЕК														
15	СУДОРОЖНЫЕ ПРИСТУПЫ														
16	ГИДРОЦЕФАЛИЯ														
17	ПЕРЕЛОМЫ														
18	НАЛИЧИЕ УСТРОЙСТВ ДЛЯ ПОДДЕРЖАНИЯ ЖИЗНЕДЕЯТЕЛЬНОСТИ														
19	АНЕМИЯ														
20	ЗАБОЛЕВАНИЯ СУСТАВОВ														
21	ПРОЛЕЖНИ														
22	ЗЛОКАЧЕСТВЕННЫЕ ОПУХОЛИ														
23	НЕДОСТАТОЧНОСТЬ ПИТАНИЯ														
24	ПРЕДШЕСТВУЮЩАЯ ИНВАЛИДИЗАЦИЯ														
	Общая сумма баллов*														

Примечание. * — общая сумма баллов показывает совокупное бремя сопутствующих заболеваний: 0 баллов — сопутствующих заболеваний нет; 1–24 балла — сопутствующие заболевания легкой степени; 25–48 баллов — сопутствующие заболевания умеренной степени; 49–72 балла — сопутствующие заболевания тяжелой степени. В случае отсутствия данных более чем по двум показателям во избежание неправильного определения степени тяжести сопутствующих заболеваний общая сумма баллов не учитывается.

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Role of Urokinase-Type Plasminogen Activator Receptor in the Regulation of Angiogenic Properties of Sca1+ Vasculogenic Progenitor Cells

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Summary

Neoangiogenesis is the key process determining myocardial regeneration after infarction. The urokinase-type plasminogen activator receptor (uPAR) is known to play an important role in the regulation of endothelial cell function and postnatal angiogenesis. However, uPAR its involvement in the regulation of the properties of vascular progenitor cells remains poorly studied.

Aim: to evaluate uPAR expression on the surface of resident cardiac vascular progenitor cells (rcVPCs) and its impact on angiogenic cell properties in vitro as well as postinfarction cardiac vascularization.

Materials and Methods. We used immunofluorescent analysis of cryosections of a murine myocardial infarction model to characterize vessels and rcVPCs, and evaluated the angiogenic properties potential of vasculogenic progenitor cells using the «tube assay» and induction of inducing differentiation in a specialized medium.

Results. We have found that the majority of Sca-1+ rcVPCs express the urokinase receptor and endothelial cell markers on their surface and are capable of proliferation and integration into the newly formed vessels in the injured area, indicating their possible involvement in the contribution to vascularization process after infarction. After acute ischemic injury, the accumulation of vasculogenic progenitor cells (8+2 and 27+7 cells per visual field, respectively; *P*=0.032) and vascularization processes (85+11 and 166+25 capillaries per visual field, respectively; P=0.033) were observed in myocardium of uPAR-/- animals, compared with wild-type animals. Our studies demonstrated that Sca-1+ rcVCPs derived from uPAR-/- murine hearts demonstrated a reduced ability to form capillary-like structures and endothelial differentiation compared with Sca-1+ rcVCPs from hearts of wild-type mice.

Conclusion. Thus, uPAR deficiency may lead to impaired vasculogenic properties of Sca-1+ rcVCPs, which is likely due to the loss of regulatory influence of specific ligands and the ability to interact with signaling mediators such as integrins. From the viewpoint of regenerative medicine, the modulation of uPAR activity can be considered as a potential target promising approach for targeted regulation of vasculogenic progenitor cells properties and postnatal angiogenesis.

HIGHLIGHT

The urokinase-type plasminogen activator receptor is involved in the regulation of the angiogenic properties of Sca1+ vasculogenic progenitor cells.

Keywords: urokinase receptor; vasculogenic cells; vasculogenesis; angiogenesis

Conflict of interest. The authors declare no conflict of interest.

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Introduction

Myocardial infarction is characterized by massive myocardial cell death and associates with genetic, molecular and cellular alterations, leading to changes in cardiac structure and size and causing gradual loss of heart function [1–3]. Acute ischemic damage of cardiac muscle triggers reparative response with the formation of a new vascular network and restoration of tissue perfusion being an integral component of it. The classical mechanism of vasculogenesis, i.e., new vessel formation from circulating endothelial progenitor cells from bone marrow, has been shown to contribute minimally to the revascularization of the damaged myocardium [4, 5]. Formation of new vessels occurs exclusively due to the endogenous pool of endothelial cells and resident cardiac vasculogenic progenitor cells (rcVPCs) present in the myocardium [4]. Several research groups have isolated and characterized cardiac-derived rcVPCs based on the expression of the Sca-1 surface marker. This population of cardiac cells is characterized by lack of expression of endothelial (CD31) and hematopoietic markers (CD45), they exhibit a profile of mesenchymal cell surface markers (CD34-, CD29+, CD90+, CD105+ and CD44+) and have been reported to be capable to differentiate toward endothelial and smooth muscle cells [6–8]. Despite a long history of studying these cells, the precise mechanisms controlling the vasculogenic behavior of rcVPCs remain poorly understood.

This study suggested that the urokinase-type plasminogen activator receptor (uPAR) may be involved in the regulation of rcVPC status. uPAR is anchored in the membrane via the GPI anchor, which ensures its mobility within the membrane bilayer and allows local concentration of urokinase proteolytic activity toward the cell movement. The cascade of proteolytic reactions triggered by urokinase, including local formation of plasmin and activation of matrix metalloproteinases, promotes the destruction of the extracellular matrix in the path of the moving cell, the activation of growth factors, and the release of growth factors sequestered in the matrix [9–11]. However, in addition to the activation of extracellular proteolysis, most cellular responses modulated by the urokinase system require transmembrane signaling, which is mediated by the interaction of uPARs with intermediary proteins that provide signal transmission via intracellular pathways regulating cell status.

The aim of the study was to evaluate the expression of uPAR on the surface of rcVPCs and uPAR impact to the in vitro angiogenesis and postinfarction cardiac vascularization.

Material and Methods

Animals. Male C57BL/129 mice (wild-type) and uPAR gene knockout mice (uPAR-/- mice) [12], provided on a free-of-charge basis by the Faculty of Fundamental Medicine of the Lomonosov Moscow State University, were used in this study. Animal genotyping was performed by PCR in accordance with the protocol of the developer company. The experiments were approved by the ethical committee of Cardiology Research Medical Center.

Myocardial infarction modeling. Experimental myocardial infarction was induced according to the protocol described earlier [13]. There were 15 mice in each group (C57BL/129 (wild-type) and uPAR gene knockout (uPAR-/- mice)).

Vessel detection in the murine myocardium. Cardiac cryosections were fixed in 4% paraformaldehyde solution, washed in PBS buffer solution, stained with antibodies to von Willebrand factor (vW) (BD, USA) for 1 h, then washed and stained with antibodies conjugated with Alexa Fluor 594 (Invitrogen, USA). For the detection of smooth muscle alpha-actin, additional staining with antibodies conjugated with FITC dye (Sigma) was performed. Characterization of Sca-1+ rcVPCs was performed by staining the samples with antibodies to Sca1 (Biolegend, USA), uPAR (Santa Cruz, USA), CD34 (Abcam, USA), CD34 (Abcam, USA) markers for 1 h, then washing and staining with antibodies conjugated to Alexa Fluor 488, 594 (Invitrogen, USA). Cell nuclei were stained with DAPI (Sigma, USA). Vessels morphometric analysis was performed by counting the number of vW+ capillaries and vW+Sca1+ vessels per visual field using the Image J software (NIH, USA).

Sca-1+ rcVPCs culture establishment. To obtain Sca-1+ rcVPCs, murine hearts (Wt and uPAR-/-) were removed from the thoracic cavity, washed in Krebs-Ringer solution with heparin, transferred into enzymatic solution (collagenase A (Roche), working concentration 1 mg/ml), and incubated in a Hybaid (Thermo Scientific, USA) shaker 2 times for 30 min at 37°C. After that, hearts were withdrawn, 5 ml of enzyme inactivation medium was added to the obtained cell suspension, and centrifuged at 300 g for 10 min. The precipitate was resuspended in the IMDM medium containing 2% fetal calf serum, B27 supplement, 20 ng/ml EGF and 40 ng/ml bFGF and transferred to gelatin-coated cups. The next day, the resultant cell culture was used to perform immunomagnetic selection using commercial selection kits and MS columns from Miltenyi biotec. At the first stage, hematopoietic cell depletion was performed using «Lineage Cell Depletion Kit» and then positive selection of Sca-1+ rcVPCs using the Cardiac Progenitor Cell Isolation Kit (Sca-1) was completed. Immunomag-

netic selection was performed according to the reagent kit manufacturer's recommendations.

Formation of capillary-like structures of Sca-1+ rcVPCs on Matrigel surface. The ability of Sca-1+ rcVPCs to endothelium-like behavior was evaluated using the model of formation of capillary-like structures on Matrigel surface. Cooled Matrigel (BD Bioscience, USA) in 350 μL volumes was applied to a 24-well culture plate and incubated for 1 h at 37°C until complete polymerization to gel texture. Cells were removed from the culture plates using Accutase solution and resuspended in EGM-2 (Endothelial Cell Growth Medium-2) supplemented with VEGF (10 ng/ml). Endothelial cells cultured in EGM-2 supplemented with VEGF (10 ng/ml) were used as a positive control. The cells were plated in wells with Marigel in the number of 80,000 per well. After 5 hours, the cells were fixed with 1% formalin solution. Microphotographs of 5 randomly selected fields in each well were obtained using an Axiovert 200M inverted microscope (Zeiss, Germany). Image J software (NIH, USA) was used for calculations.

Differentiation of Sca-1+ rcVPCs into endothelial cells. To induce cell differentiation in the vascular direction, the differentiation medium described earlier [14] was used (DMEM/F12 supplemented with 10% fetal calf serum, insulin-transferrin-selenite, and 10 ng/ml VEGF). The differentiation medium was replaced every 24 hours. The cells were cultured for 14 days. Endothelial differentiation was tested by immunofluorescent staining of cells with von Willebrand factor antibodies (DAKO, USA) and secondary antibodies conjugated with fluorescent labeling. Quantification of cells differentiated in the endothelial direction was performed using Image J software (NIH, USA).

Microscopy and image analysis. Myocardial cells and cryosections were analyzed using an Axiovert 200 M fluorescence microscope (Carl Zeiss, Germany) and AxioVision 3.1 software (Carl Zeiss, Germany).

Statistical analysis. The normality of the data distribution was assessed using the Kolmogorov–Smirnov test. Significance of differences between the samples was assessed using Mann–Whitney *U*-criterion. Statistical analysis of the results was performed using Statistica 8.0 software (StatSoft, Inc.). Data were presented as mean±standard deviation (*M*±*SD*).

Results

On day 5 post myocardial infarction, there was a 2-fold decrease in the total number of capillaries in uPAR-/- animals in the necrotic area (Fig. 1, *c*), compared with wild-type animals (85+11 and 166+25 capillaries per visual field, respectively; *P*=0.033), indicating impaired myocardial vascularization after acute ischemic injury.

Taking into account the identified differences in the formation of vessels, the content of vasculogenic progenitor cells which may participate in this formation, was analyzed. Compared with wild-type mice, a 3-fold decrease in the number of Sca-1+ progenitor cells (Fig. 1, *a, d*) (27+7 and 8+2 cells per visual field, respectively; *P*=0.032) that can differentiate in the vascular direction and release proangiogenic growth factors was observed in uPAR-/- animals [6]. Most Sca-1+ rcVPCs expressed the urokinase receptor on their surface (Fig. 1, *b*) and were characterized by a lack of hematopoietic cell markers (CD34 and CD45), which precludes their bone marrow origin. We found that some Sca-1+ rcVPCs co-localized with endothelial cell markers (CD31, vW) and were part of the newly formed vessels in both the necrosis zone and the peri-infarct area.

In view of the identified signs of reduced vascularization of the injured zone in uPAR-/- animals, we conducted experiments to evaluate the angiogenic properties of these cells in vitro (Fig. 2).

Our studies showed that Sca-1+ rcVPCs obtained from the hearts of uPAR-/- mice showed a reduced ability to form capillary-like structures compared with Sca-1+ rcVPCs from the hearts of wild-type mice (Fig. 2, *a, b*). Morphometric calculations showed (Fig. 2, *c, d*) that the total length of vascular structures (57969+6998 (Sca1+uPAR-) and 83302+6464 (Sca1+Wt) relative units; *P*=0.037) and their branching capacity (1900+397 (Sca1+uPAR-) and 3322+501 (Sca1+Wt); *P*=0.036) was reduced in Sca-1+ rcVPCs compared with control cells. The impaired endothelial-like behavior associated with impaired endothelial differentiation ability of Sca-1+ rcVPCs induced by the dedicated medium (Fig. 3). Culturing Sca-1+ rcVPCs (from uPAR-/- and wild-type murine hearts) promoted the formation of capillary-like structures. Meanwhile, the ability of Sca-1+ rcVPCs obtained from uPAR-/- hearts to form vWF+ vascular structures was 5 times lower compared to cells from wild-type hearts (7+4 (Sca1+uPAR-) and 33+17 (Sca1+Wt) von Willebrand+ cell structures per visual field; *P*=0.01) (Fig. 3).

Discussion

Our research show that some Sca-1+ rcVPCs express endothelial cell markers on their surface and are capable of proliferation and integration into the newly formed vessels in the injured zone, indicating their possible participation in vascularization after infarction. Urokinase receptor was found on the surface of most Sca-1+ rcVPCs, which can participate in the regulation of rcVPC function through interaction with urokinase and/or vitronectin [15, 16]. When uPARs interact with specific

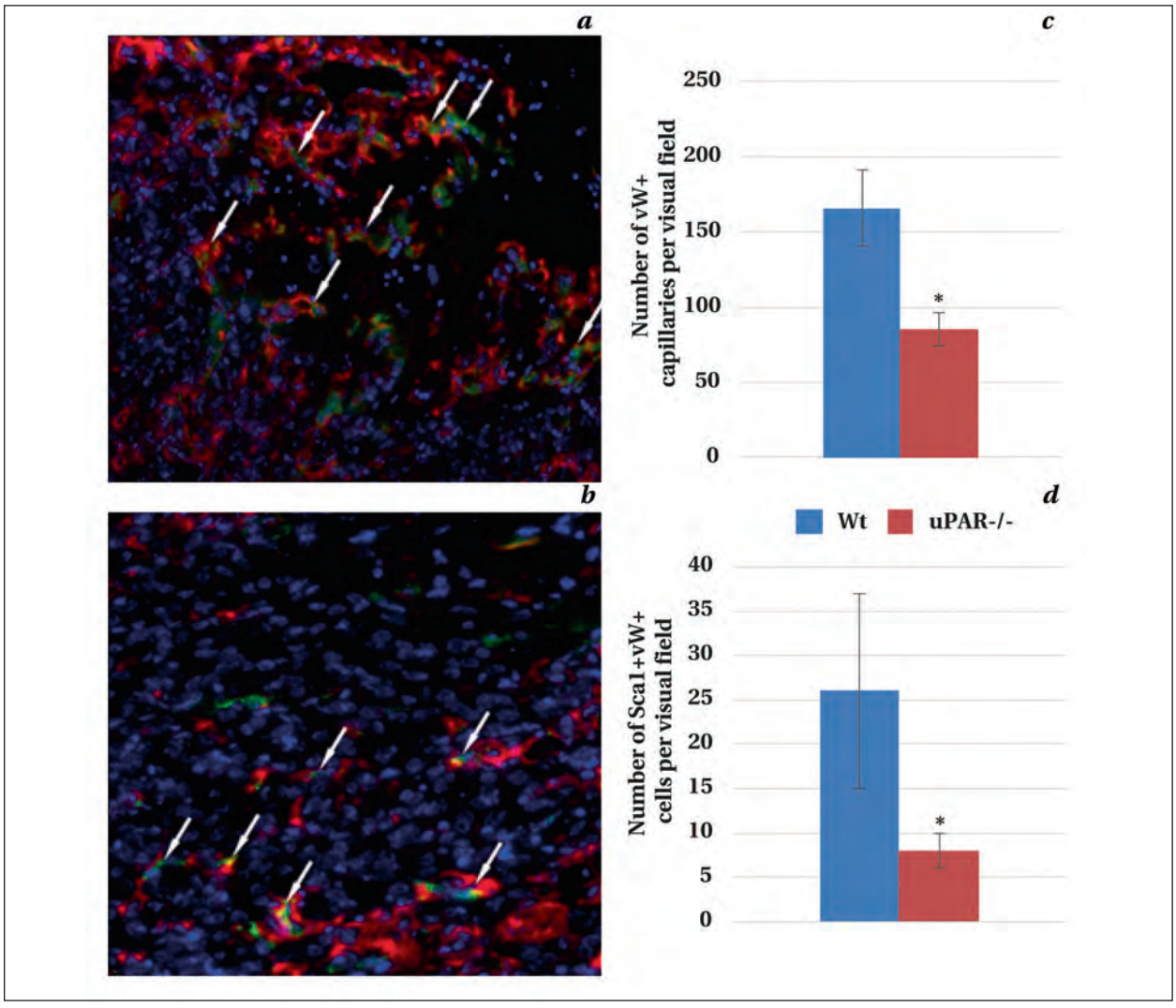

Fig. 1. Sca1+ resident cardiac vasculogenic progenitor cells express von Willebrand factor (vW) and urokinase receptor (uPAR). **Note.** *a* — vW (red), Sca1 (green). *b* — uPAR (red), Sca1 (green). Yellow staining indicates co-localization of signals. c — quantitative assessment of capillary content in the zone of postinfarction necrosis (day 5 after myocardial infarction) in wild-type and uPAR-/- mice. *d* — quantitative assessment of Sca1+vW+ capillary content in the zone of postinfarction necrosis (day 5 after myocardial infarction) in wild-type and uPAR-/- mice. For Fig. 1–3, data are presented as mean±standard deviation (*M*±*SD*). * — $P<0.05$.

ligands [17–19], intracellular signaling cascades are activated, promoting adhesion, cell proliferation, and vascular differentiation [20–22]. Consequently, this kind of interaction might serve as a potential stimulus for the regulation of rcVPC functions. Indeed, reduced accumulation of Sca-1+ vasculogenic progenitor cells and impaired postinfarction vascularization were observed in the heart of uPAR-/- animals after acute ischemic injury compared with wild-type animals. In addition, Sca-1+ rcVPCs derived from uPAR-/- mice hearts exhibited reduced endothelium-like behavior (formation of capillary-like structures) and angiogenic differentiation in vitro, compared with Sca-1+ rcVPCs from wild-type mouse hearts. Disrupted interactions between uPARs and integrins result in the suppression of the activity of Rac and Cdc42 minor Rho GTPases, thereby inhibiting their participation in the rearrangement of the cytoskeleton and cell motility, which leads to the loss of the cells' ability to integrate into the forming vessels and participate in vasculogenesis [23]. The revealed changes are similar to the ones observed in vasculopathy in patients with systemic scleroderma, where uPAR cleavage between the first and second domains by MMP12 is seen [24, 25]. Overproduction of MMP12 in endothelial cells and uPAR dysfunction led to suppression of uPA-induced migration, invasion, proliferation of vascular cells and formation of capillary-like structures on matrigel surface [26, 27]. Moreover, adding the anti-MMP-12 monoclonal antibodies promoted recovery of endothelial cell angiogenic activity including ability to migrate, invade and form vascular structures [24, 27]. Furthermore, the same researcher

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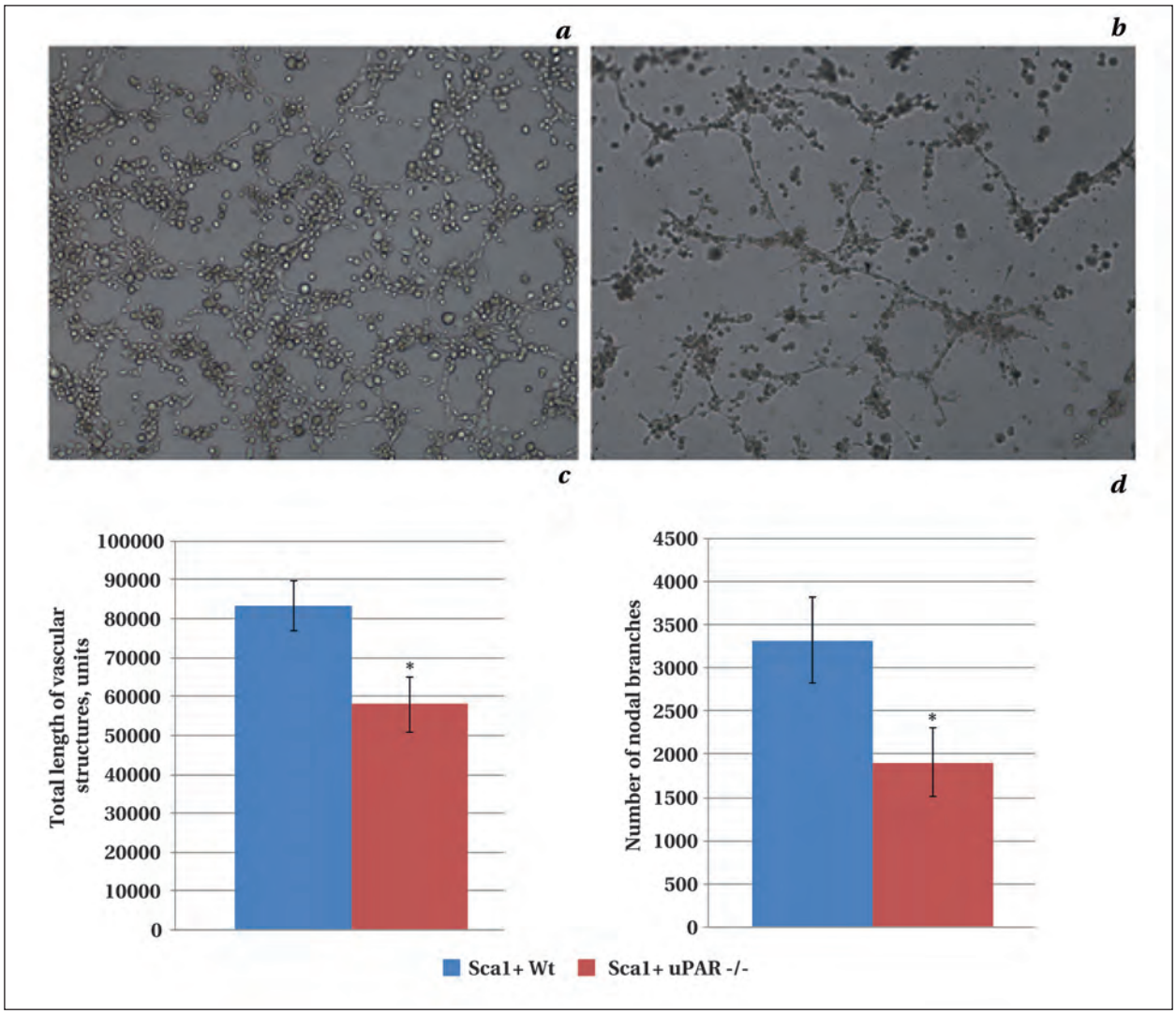

Fig. 2. Representation of the ability of Sca-1+ rcVPCs isolated from wild-type murine hearts (*a*) and uPAR-/- (*b*) to form capillary-like structures in vitro.
Note. *c, d* — quantitative assessment of the total length of vascular structures formed by Sca-1+ rcVPCs from the hearts of uPAR-/- and wild-type mice and their branching ability.* — $P<0.05$.

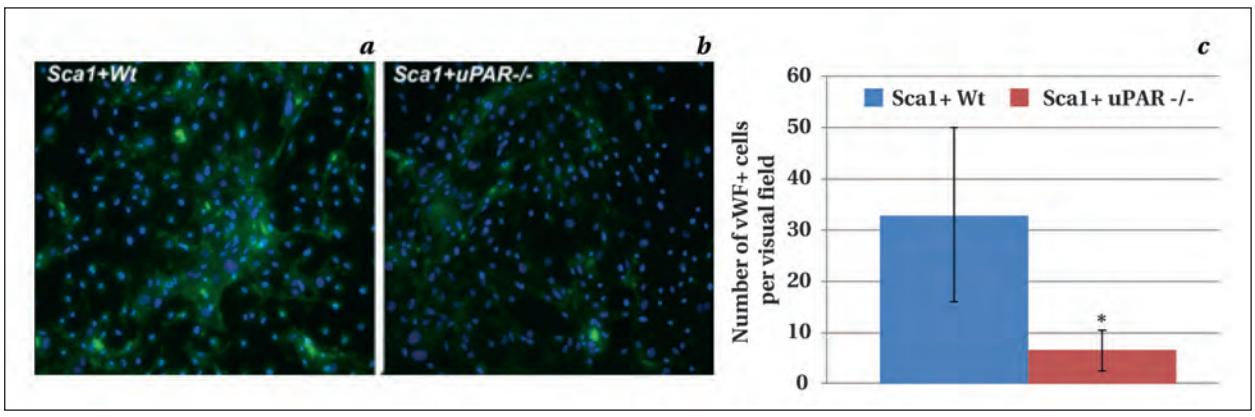

Fig. 3. Representation of the differentiation ability of Sca-1+ rcVPCs isolated from the hearts of uPAR-/- and wild-type mice.
Note. *a, b* — staining of Sca-1+ rcVPCs with antibodies to von Willebrand factor (vWF) (endothelial cell marker) (green) after cultivation in differentiation medium. *c* — quantification of the number of vWF+ structures after cultivation in differentiation medium. * — $P<0.05$.

team showed that uPAR cleavage in endothelial cells in systemic scleroderma leaded to loss of integrin-mediated uPAR binding to the actin cytoskeleton [28–30] thus abrogating a key step in vascular formation.

Conclusion

The uPAR deficiency leads to impaired vasculogenic properties of Sca-1+ rcVPCs, which is probably associated with the loss of regulatory effect of specific ligands and the ability to interact with signaling mediators, such as integrins. From the viewpoint of regenerative medicine, modulation of uPAR activity can be considered as a potential target for specific regulation of the vasculogenic progenitor cells functions and postnatal angiogenesis.

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Conservative Therapy of Pressure Ulcers Using Physical Methods (Review)

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Summary

In Russia and European countries, the incidence of pressure (decubitus) ulcers in sedentary patients with severe comorbidities, spinal trauma, and cerebral accident sequelae varies from 3 to 40%, reaching 80% in the chronic critical illness. The final result of conservative treatment of pressure ulcers (PU) with adequate correction of comorbidities and proper care of the immobilized patient depends largely on the choice of local interventions.

Aim of the review: to demonstrate the range of effective methods of physical treatment of pressure ulcers suitable for conservative treatment.

Materials and methods. We selected and analyzed 80 scientific publications. Sources were selected from the PubMed, Scopus, and RSCI databases of medical and biological publications. The following methods were covered: negative pressure wound therapy, ultrasonic methods, hydrosurgical treatment, plasma flows, laser therapy (including low-level laser therapy), «hardware» acceleration of regenerative processes, including electrical stimulation, as well as a combination of these methods.

Results. The variety of physical methods of wound treatment, on the one hand, provides an opportunity to select an individual therapy program. On the other hand, each method has its own limitations and contraindications. That is why in practice various combinations of these methods are reasonable.

Conclusion. For several objective reasons, no reduction of PU incidence in clinical practice is expected. Therefore, studying the issues of evidence-based clinical effectiveness and economic feasibility of various conservative techniques of decubitus treatment to find best solutions in this area is warranted.

Keywords: pressure ulcer; bedsore; surgical technology; physical treatment

Conflict of interest. The authors declare no conflict of interest.

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Introduction

Bedsores, or decubitus ulcers (DU), are one of the major health care problems worldwide. They affect patients with severe comorbidities, spinal trauma, and cerebral accidents. In Russia and European countries, the incidence of DU in sedentary patients with the above-mentioned comorbidities varies from 3 to 40%, reaching 80% in chronic critical illness due to severe brain damage [1–5].

In immobilized patients, it is extremely difficult to completely avoid factors contributing to DUs (e. g., constant high pressure on the skin and soft tissues, friction and body movements, moist skin), which complicates their treatment [1, 6–9]. In addition, the severity of the underlying disease entails depletion of plastic reserves of the patient's body, which is usually followed by anemia, pneumonia, and protein-energy deficiency [10–13]. All the above mentioned conditions dramatically slow down the wound healing, sometimes for many months, and due to heterogeneity of macroscopic changes one can simultaneously observe areas of necrosis and granulation tissue [14, 15].

When radical surgical correction of DU is not feasible, prolonged conservative therapy is the only treatment option [16, 17]. Comprehensive approach including conservative measures aimed at stabilizing the patient's status, eliminating the source of external pressure, correct positioning in bed, and active surgical ones, such as debridement and drainage of the infection focus [8, 17–24], should be employed [8, 17–24]. The use of new physical treatment methods, up-to-date wound dressings, and pharmacological formulations to create optimal

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conditions for the healing of the wound defect are crucial in this situation [17, 25–38]. Undoubtedly, the success of conservative therapy directly depends on the size of the decubitus ulcer; however, some authors have reported cases of successful treatment of stage III–IV DU during 4–6 weeks with the appropriate addition of methods stimulating tissue repair [17, 24, 39, 40].

The aim of the review is to show the range of effective physical treatment methods for decubitus ulcers suitable for conservative treatment.

To evaluate the physical methods, we searched the English-language PubMed and Scopus databases of medical and biological papers, as well as the Russian RSCI bibliographic system for publications dated within the last 20 years.

General description of physical treatment methods

A separate vast area in conservative therapy of chronic non-healing wounds, including DUs, involves topical treatment with physical and chemical agents. In this case, a particular method can be used not only alone, but also as a part of an integrated treatment, ensuring adequate forced necrolysis (cleaning decubitus ulcers from fibrin, pus and dead tissues), stimulating regeneration and wound epithelialization [4, 14, 17, 41].

Physical factors of mechanical and wave energy. Vacuum therapy of wounds based on negative pressure generation in the DU area is currently widely available [42–45]. Its therapeutic effect is achieved not only by forced cleaning of the lesion from bacteria and necrotized tissues, but also due to normalization of pH of the wound environment, enhancement of microcirculation in the wound area and lymph flow, control of edema and exudation. There are also biological effects such as stimulation of granulation tissue, microdeformation and reduction of inflammation [46]. This method is effective as a part of an integrated treatment of purulent and necrotic soft tissue lesions, including DU, fistulas, trophic ulcers, peritonitis. In addition, vacuum therapy is successfully used in highly contaminated DUs (e. g., as a result of fecal contamination) [47]. Advances in technology and development of portable negative pressure generators have promoted this technology [25, 26, 45]. Recently, researchers suggested using vacuum therapy with fluid (such as normal saline) instillation (NPWTi-d) to increase the efficiency of the procedure [48, 49]. Such a combination improves wound cleansing and accelerates granulation tissue formation.

The use of ultrasound is one of the well-established methods. The therapeutic efficacy of this technique is based on the destructive effect of oscillating waves without a significant increase in tissue temperature [50]. Two ultrasound treatment types used in DU therapy include ultrasonic blade debridement and ultrasonic cavitation [50]. The latter causes destruction of non-viable tissues containing much fluid or calcifications due to formation of microbubbles in liquid medium under the effect of high amplitude ultrasound wave [28, 51]. Meanwhile, «selective destruction» is performed, because healthy tissues contain a large number of elastic elements such as vessels and ducts which are less responsive to the ultrasonic wave. In addition, metabolic shifts generated during ultrasonic cavitation trigger a wide range of photochemical reactions, which, for example, cause mast cell degranulation, increased prostaglandin activity, disintegration of bacterial cell membranes [50]. The bactericidal effect of ultrasonic cavitation can be enhanced by using an antiseptic solution acting as an acoustic medium [28, 50]. Moreover, the use of non-contact devices with relatively constant settings can reduce the inflammatory response despite the lack of significant changes in the bacterial load. Ultrasonic devices requiring direct contact with the wound decrease bacterial load by reducing the biofilms [51]. Thus, the physician can choose which component of the infectious inflammation needs more attention at a specific stage of treatment.

Mechanical debridement of pressure ulcers also includes hydrosurgical treatment, which allows to selectively remove bacteria and nonviable tissues from the decubitus avoiding damage to the surrounding viable tissues [52]. A high-speed (up to 1600 km/h) fine-dispersed flow of normal saline is generated in the device under ultra-high pressure. The jet movement is tangential to the wound. Like a scalpel blade, it cuts necrotic tissue, fibrin deposits, preserving surrounding viable tissues [53]. Thus, a clean and smooth surface of the decubitus is quickly formed. This intervention, however, requires general anesthesia.

Physical factors of thermal energy include laser radiation and plasma technology. Over the past 15–20 years, plasma flows (PF) have become widespread in clinical practice. The studies have shown such advantages of the technology as virtually bloodless dissection of necrotic tissue, high quality hemostasis due to high-energy exposure, wound surface sterilization due to «hard» ultraviolet irradiation (λ<250nm), high ozone concentration (>0.5 mg/m^3) in the operating field [54]. More importantly, optimal conditions are created for tissue regeneration primarily due to the stimulating effect of intermediate ionization products and exogenous nitric oxide (II) of the air-plasma jet (NO-therapy). According to A. Shulutko et al. (2018), the use of plasma «scalpel» in 290 patients with purulent necrotic soft tissue lesions reduced the number of surgical debridement sessions by 1.5–2 times (P<0.05) with the significant reduction of intraoperative blood loss [55]. As a result of va-

porization of necrotized areas, purulent wound transforms into a predominantly burn wound, which accelerates the healing process (duration of all phases of the wound healing decreases by 1.5–1.8 times) and increases the likelihood of favorable outcome. Qualitative changes were also confirmed by cytological and histological examination. The non-contact character of procedures in all modes, absence of any side effects, full «compatibility» with topical medications, various wound dressings and other physical treatment methods should be considered undeniable advantages of PF [54].

In recent years, the use of laser energy in surgery has been the most popular due to laser-induced wide range of optical and photobiological effects. Both surgical and therapeutic laser modes are used in the treatment of DU. In the contaminated surgery, YAG-Ho-, CO2-, and YAG-Nd-laser systems have proved to be highly effective [56]. Due to the superthermal effect, reliable hemostasis, high quality debridement and high-energy necrolysis of the ulcerated wound surface (vaporization effect), as well as total decontamination of DU are provided. As a result of these effects, the duration of suppurative inflammation phase significantly decreases [57]. The use of a high-power and high-density laser beam is indicated for debridement [58].

Low-energy laser technology (LELT) is now successfully used for local treatment of pressure ulcers, as well as chronic non-healing purulent wounds and trophic ulcers [59–62]. The therapeutic efficacy of this technique is due to its positive effect on energy metabolism, decrease in the intensity of peroxidation, normalization of pH of the wound environment, improvement of tissue regenerative potential, lymph circulation and microcirculation in the area around the wound [61, 63] Specific biochemical mechanisms of the positive effect of LELT have been shown in the study by J. Taradaj et al. (2018) who reported expression of anti-inflammatory cytokines and increased concentration of angiogenesis factors induced by low-energy laser [62]. In addition, an antibacterial effect due to the free radical and reactive oxygen species production in the target area can be observed, which helps to combat antibiotic resistance [64]. Even a local immunomodulating effect of the technique has been noted. Thus, in the paper by M. Brauncajs et al. (2018), the anti-inflammatory effect of LELT was demonstrated when, after comparing the results of 6 patients who received a course of laser stimulation, a significant decrease in the level of protein cathelicidin LL-37 (chemoattractant for immune cells) in DU tissues was noted [65]. The authors emphasize the immunomodulatory and indirect antimicrobial properties of the technique. The use of various modes of laser energy in combination with other conservative methods allows more complete and faster preparation of deep decubitus for plastic intervention [60].

Several publications on the use of therapeutic laser in the treatment of pressure ulcers have been published recent years. Thus, S. Palagi et al. (2015) described a case report of successful conservative treatment of a patient using modern wound dressings and LELT, which reduced the size of a large decubitus ulcer from 7×6 cm to 1.5×1.1 cm [56].

Some authors suggest combining the therapeutic laser treatment with other physical methods described earlier (magnetotherapy, photodynamic treatment, ultrasonic cavitation, etc.) [28, 59]. In particular, L. Rosa et al. (2017) succeeded in achieving complete epithelialization of a deep decubitus of the heel area in an 82-year-old diabetic woman using a combination of photodynamic therapy, LELT and topical cellulose-based membranes for wound healing [66].

In a review by R. Machado et al. (2017), all known laser studies concerning DU were analyzed with a total of 386 publications found in Medline, PEDro, Cochrane, and CENTRAL databases [59]. The 4 selected and analyzed papers described LELT variations with various wavelengths (658, 808, 904, and 940 nm). The authors of the review concluded that the most significant clinical effect can be expected with regular treatment of DU with a monochromatic wave of 658 nm for 1 month. Positive experience of the use of LELT to treat various types of chronic wounds can be found in the earlier publications of Russian specialists [54, 55].

Device-based methods for accelerating the regeneration. Being the most extensive and heterogeneous group in terms of technology, these methods are widely used in phase 2 of the complicated wound process in DU. They include high-frequency low-power impulse therapy, magnetic therapy, photodynamic therapy, periodic exposure to direct electric current, hyperbaric oxygenation, ozone therapy, phonophoresis with antiseptics, ultraviolet irradiation in suberythemic doses, electrophoresis with antibacterial and anti-inflammatory drugs, treatment of pressure sores with pulsating jet [67–75]. The last three techniques have also proven to be beneficial in controlling inflammation around the wound. Although data on efficacy of individual techniques were reported in 2016–2020, the Cochrane reviews do not always confirm this positive effect. The reason may lie in methodological limitations, lack of quality fundamental works, insufficient number of observations, and/or incorrect study design. This is especially relevant for various techniques of electromagnetic stimulation and local thermal treatment of decubitus [69, 74]. Thus, when evaluating the effect of high amplitude pulsed current on decubitus ulcer,

the authors concluded that despite the positive effect of reducing the area of pressure ulcers when using complex therapy, the data needed to be confirmed by more high-quality studies due to the small number of included research trials [76]. In most studies, the assessment of effectiveness of physical methods and comparative analysis were performed on small samples (up to 40 cases). Whereas some researchers are cautiously positive about efficacy of electrostimulation for healing of pressure ulcers, but the level of evidence is not high enough for definitely recommending it to treat decubitus ulcers [77].

Combination techniques. Researchers are increasingly aware that monotherapy for treating chronic non-healing wounds has limited efficacy, so combinations of different local treatment techniques are proposed. The combination of vacuum therapy, ultrasound cavitation and hydrosurgery («VivanoTec» system, «Sonoca-180» machine and «Versajet» system, respectively) showed good results in the treatment of stage III–IV DU in 49 patients with spinal trauma (S. Shapovalov et al., 2016). The use of this combination approach reduced the treatment period by 3 times and thus accelerated the preoperative preparation for decubitus defect reconstruction [53].

Yu. Tsupikov (2007) described the results of the combined technique of DU management based on the combination of interactive or specialized wound dressings and local ozone therapy. According to the authors, this technique provides 1.3 times shorter healing time and 1.5 times shorter preparation time for reconstruction of pressure ulcers [78]. Similar studies can be found in the international literature [79]. W. Baek et al. (2020) based on statistical analysis of a small prospective sample (n=38) indicated a significant (P=0.001) acceleration of DU reduction with vacuum therapy (NPWT) and lipid-colloid wound dressings when compared with NPWT alone [26].

In 2013, A.Struchkov et al. proposed triple-component combined ozone therapy, which includes intravenous injection of ozonized saline, daily irrigation of the wound surface with an ozone-oxygen gas mixture and subcutaneous injection of the edges of the decubitus defect with this mixture. According to the authors, this technique promotes the healing of stage II and small stage III decubitus ulcers without surgical intervention in 79% of patients [67]. Furthermore, continuation of local ozone therapy sessions in the postoperative period was found to also reduce the risk of DU recurrence.

H. Duan, H. Li et al. evaluated a noteworthy combination of surgical debridement followed by extracorporeal shockwave therapy with an alginate dressing to treat stage IV DU in a patient with posthypoxic encephalopathy. The authors have emphasized efficacy and safety of this combination which could be useful for elderly patients with decubitus [80].

Conclusion

The problem of the efficient medical care for patients with pressure ulcers is still far from being resolved. In addition, due to objective reasons, we cannot expect the incidence of decubitus ulcers to decrease in clinical practice. The outcome of the conservative treatment of decubitus ulcers, with adequate correction of underlying diseases and proper care of an immobilized patient, largely depends on a local treatment. On the one hand, a variety of physical methods of wound treatment gives an opportunity to choose an individual program of DU therapy. On the other hand, each method has its limitations and contraindications, while the techniques used are not always evidence-based, clinically efficient or cost-effective. Therefore, studying and addressing the above-mentioned issues in the conservative treatment of pressure ulcers is warranted.

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Based on the **«Brief author guidelines for preparing and formatting scholarly papers in journals indexed in international scientific databases»** edited by Olga Kirillova under the ASEP (Association of Scientific Editors and Publishers) and RRIEPL (Russian Research Institute of Economics, Politics and Law in Science and Technology) published in 2019, **The CSE's White Paper on Promoting Integrity in Scientific Journal Publications**, 2012 Update, **ICMJE Recommendations for the Conduct, Reporting, Editing and Publication of Scholarly Work in Medical Journals** (December 2016), and **The European Association of Scientific Editors (EASE) Guidelines for Authors and Translators** (available at **https://ease.org.uk/guidelines-toolkits/**).

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