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# ELECTROKINETIC, OXIDATIVE AND AGGREGATION PROPERTIES OF RED BLOOD CELLS IN THE POSTOPERATIVE PERIOD FOLLOWING KIDNEY TRANSPLANTATION

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**Objective:** to study the electrokinetic and aggregation properties, as well as the pro-oxidant and antioxidant processes in red blood cells following kidney transplantation in donors and in recipients in the postoperative period. **Materials and methods.** Blood from 12 recipients and 5 kidney donors over time – before transplantation, as well as at week 1, months 1, 2, 7, 10 and 12 after surgery, as well as from 8 healthy volunteers who formed the control group. We used microelectrophoresis to measure the electrophoretic mobility of red blood cells, characterizing the electrokinetic properties of cells. Aggregation was calculated microscopically by counting unaggregated red blood cells. Malondialdehyde concentration was measured spectrophotometrically at its absorbance maximum at 530 nm by reaction with thiobarbituric acid. Catalase activity was analyzed by reducing hydrogen peroxide in the sample spectrophotometrically at 240 nm wavelength. The obtained values were compared using the Mann–Whitney U test. **Results.** Decreased electrophoretic mobility of red blood cells within 2 months after transplantation was associated with increased malondialdehyde concentration and erythrocyte aggregation, decreased catalase activity in kidney recipients, followed by restoration of indicators to the control values. Electrophoretic mobility of red blood cells decreased, while malondialdehyde concentrations increased in donors after surgery. However, the increase was less pronounced than in recipients. The changes indicate that the postoperative period causes changes at the cellular level both in donors and in recipients. This is manifested by decreased stability of erythrocyte membrane structure, which is largely determined by lipid peroxidation processes. At the systemic level, a change in the electrophoretic mobility of red blood cells indicates a stress reaction before and after kidney transplantation in recipients within 2 months after surgery, and in donors in 1–2 months in the postoperative period with gradual increase in the body's resistance. **Conclusion.** Kidney transplantation is manifested at the cellular and systemic levels. At the cellular level, there is decreased stability of the membrane structure, which is largely determined by lipid peroxidation processes. At the systemic level, a change in the electrophoretic mobility of red blood cells indicates a stress reaction with gradual increase in the body's resistance. The data obtained demonstrate changes in the functional properties of red blood cells both in kidney transplant recipients and in donors. These changes need to be taken into account when carrying out therapeutic measures.

*Keywords: kidney transplantation, red blood cells.*

## INTRODUCTION

Kidney transplantation is the best modality of renal replacement therapy for patients with irreversible acute and chronic kidney diseases [1]. The life expectancy of patients with a transplanted kidney exceeds that of those treated with hemodialysis and peritoneal dialysis. It provides a high quality of life [2, 3]. However, organ transplantation continues to be a complicated surgical procedure with the risk of developing several major complications [4]. Cardiovascular disease is the most common cause of morbidity and mortality in patients with a transplanted kidney [5]. Incidence of acute rejection crises, arterial hypertension, proteinuria, hyperglycemia, and anemia are also considered as risk factors for allograft dysfunction [6–8]. Besides, arterial thrombosis and venous thrombosis are distinguished, which in most cases occur in the first week after transplantation, although they can also appear in a longer time frame [9].

It should be considered that renal toxicity, ischemia-reperfusion injury and immunological disorders of the kidney lead to increased formation of reactive oxygen species [10]. In addition, some immunosuppressants increase oxidative stress, especially compounds from calcineurin inhibitors, and thus indirectly increase the risk of complications [11–12]. Impaired oxidative balance plays a huge role in the patient's homeostasis. Pathogenesis of

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arising and developing disorders in this case includes oxygen deficiency, activation of free radical oxidation – lipid peroxidation stimulation, which leads to changes in the structure and function of cell membranes, damage to cellular and subcellular structures, and aggravation of the pathological state [13, 14]. Metabolic, circulating and hemodynamic factors have a greater impact on the functional state of the kidney graft of the post-traumatic period [15].

The functional state of red blood cells of kidney donors and recipients in the postoperative period was analyzed considering that red blood cells (RBCs) play an important role in maintaining oxygen homeostasis in the body, which, if disrupted, causes tissue hypoxia with development of free radical processes. At the same time, we could not find studies on the state of red blood cells in kidney recipients. Moreover, there is scarce information on the state of donors not only at the cellular, but also at the organ level. The aim of this work was to study the electrokinetic, aggregation properties, as well as the pro-oxidant and antioxidant processes in red blood cells of kidney donors and recipients in the postoperative period.

## MATERIALS AND METHODS

The study investigated the blood of kidney recipients and donors in the postoperative period. Kidney explantation and transplantation operations were carried out at Volga Regional Medical Center, Federal Medical and Biological Agency (FMBA), where such medical interventions have been performed since 2006 [16]. All patients gave their voluntary consent in a form approved via executive order No. 517n of the Russian Ministry of Health, dated August 11, 2017. The study was approved by the local ethics committee of FMBA. The study included 12 patients aged 40 to 54 years, who underwent kidney transplantation. Five people donated their kidneys, and 8 people were healthy volunteers who made up the control group. Blood for analysis was taken from the median cubital vein over time – before surgery, at postoperative week 1, at postoperative month 1, 2, 7, 10, and 12.

Electrokinetic and aggregation properties were determined by measuring the RBC electrophoretic mobility and via optical measurements of RBC aggregation. RBC electrophoretic mobility was determined by microelectrophoresis using a cytoferometer in our modification [17]. The time it took the RBCs to travel a distance of 100 μm was recorded in a Tris-HCl pH 7.4 buffer at 8 mA current intensity. The RBC electrophoretic mobility magnitude was determined by the formula: U = S/TH, where S is the distance covered by the cells, T is the time taken by the cells to cover a distance of S, H is the potential gradient. The magnitude of the potential gradient was determined by the formula: H = I/gχ, where I is the current intensity, g is the chamber cross section, and χ is the specific electrical conductivity of the medium. RBC aggregation was studied by optical microscopy by counting single RBCs and their aggregates (Derjugina, 2006). A blue dextran T-2000 solution (GE Healthcare firm, 20 mg/mL) in a Tris-HCl buffer (pH 7.4) was used as an aggregation stimulator. The washed RBCs were diluted with a dextran solution (at a 1:10 volume ratio) and the number of non-aggregated RBCs was counted in the Goryaev's chamber. The total number of RBCs in the sample was counted in an isotonic NaCl solution. The level of aggregation (A) was calculated by the formula: A = 100% – (the number of free (non-aggregated) RBCs × 100%/total number of RBCs).

Pro-oxidant and antioxidant properties of RBCs were evaluated by malondialdehyde (MDA) levels and activity of catalase in RBCs [18]. Serum MDA levels in RBCs were determined spectrophotometrically at 530 nm absorption maximum during reaction with thiobarbituric acid. Molar extinction coefficient ($E = 1.56 \times 10^{-5}$ $M^{-1}cm^{-1}$) was used to calculate serum MDA level. Catalase activity was analyzed by reducing hydrogen peroxide ($H_2O_2$) in the sample. Measurements were carried out spectrophotometrically immediately after addition of $H_2O_2$ in the sample cuvette and 20 seconds after addition at 240 nm wavelength.

The data obtained is presented as mean values ± mean error. The obtained values were compared using the Mann–Whitney U test. Differences between groups were considered significant at ≤0.05 significance level.

## RESULTS AND DISCUSSION

Results of studies have shown that in kidney transplant recipients, RBC electrophoretic mobility was significantly reduced relative to the physiological norm within two months after surgery. It was then restored to the level of the control group (Fig. 1). It should be noted that RBC electrophoretic mobility also reduced prior to

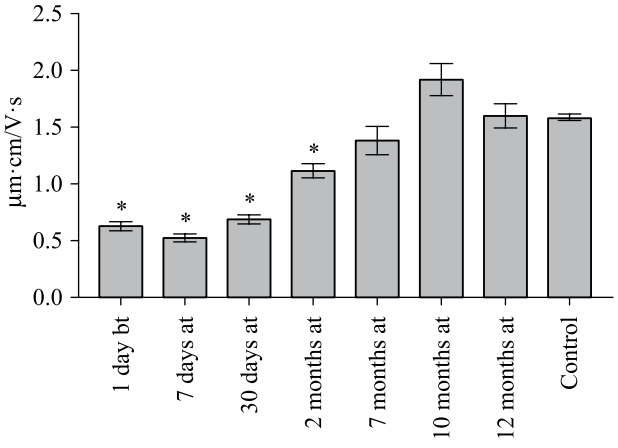


Fig. 1. Dynamics of electrophoretic mobility of erythrocytes in patients with kidney transplantation

*Note*. Here and in other figures: bt – before transplant; at – after transplant; * – statistically significant differences to control ($p < 0.05$)

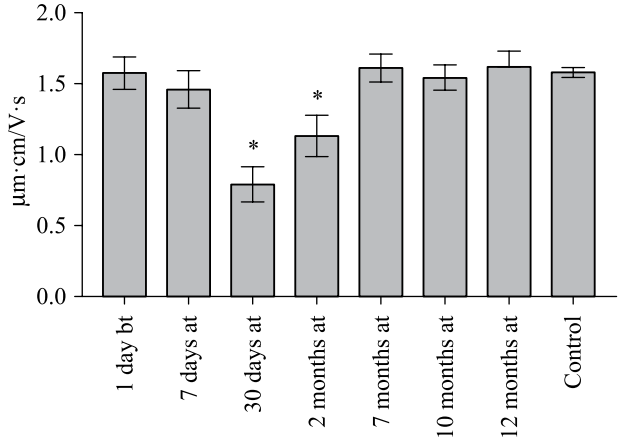


Fig. 2. Dynamics of electrophoretic mobility of erythrocytes of kidney donor

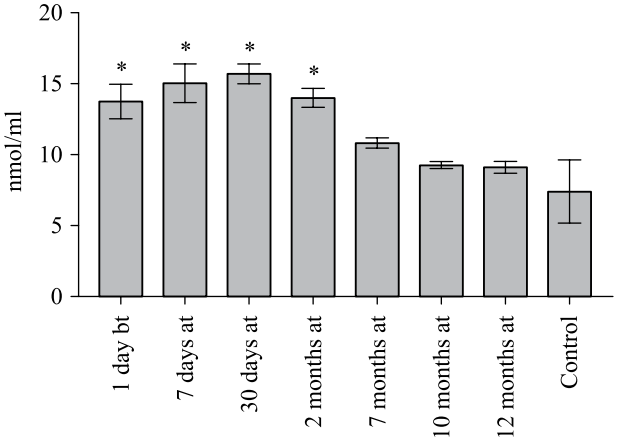


Fig. 3. Dynamics of aggregation of erythrocytes in patients with kidney transplantation

surgery. Kidney donors had reduced RBC electrophoretic mobility at postoperative month 1 and 2 (Fig. 2).

Decreased RBC electrophoretic mobility under various extreme influences and conditions is a reflection of the general nonspecific reaction of the body to a stimulus and a stress-response severity criterion [19, 20]. Analysis of RBC electrophoretic mobility reveals changes in adrenal functional activity and diagnoses of the direction of processes associated with activation or inhibition of nonspecific stress resistance of the body [20, 21]. Moreover, decreased RBC electrophoretic mobility accompanies activation of the sympathoadrenal system, whereas its increase is associated with activation of the pituitary-adrenal system and higher resistance of the body [21]. Thus, RBC electrophoretic mobility at different stages of the postoperative period reflects a stress response in recipients and donors, which is recorded before month 2, with gradual activation of stress-regulating reactions and triggering of adaptive mechanisms at month 7 after operation.

RBC surface charge determines the aggregation characteristics of cells, which have a significant role in blood flow under microcirculation conditions. A study of RBC aggregation properties revealed that kidney transplant recipients had increased RBC aggregation at month 2 of the post-transplant period ($p \leq 0.001$) (Fig. 3). In kidney donors, the RBC aggregation properties did not statistically significantly change (Fig. 4).

In turn, RBC surface characteristics are determined by the properties of cell membranes, whose state largely depends on pro-oxidant and antioxidant processes. A study of Serum MDA levels showed that in kidney transplant recipients, MDA red blood cells concentration before month 2 was significantly higher than that of the control group (Fig. 5). Subsequent measurements of Serum MDA levels from month 7 revealed no differences from the physiological norm. It was shown that in kidney donors, increased levels of MDA were observed

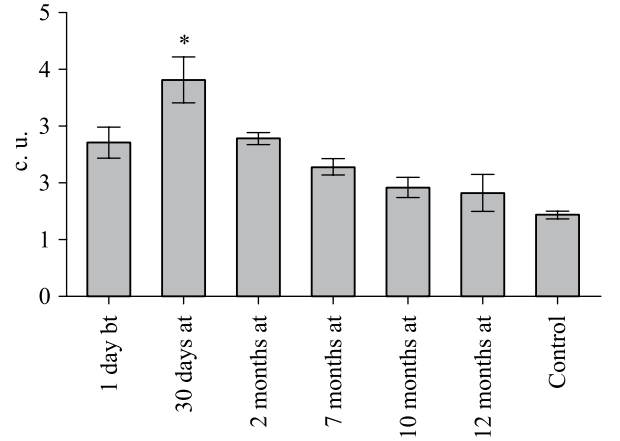


Fig. 4. Dynamics of aggregation of kidney donor erythrocytes

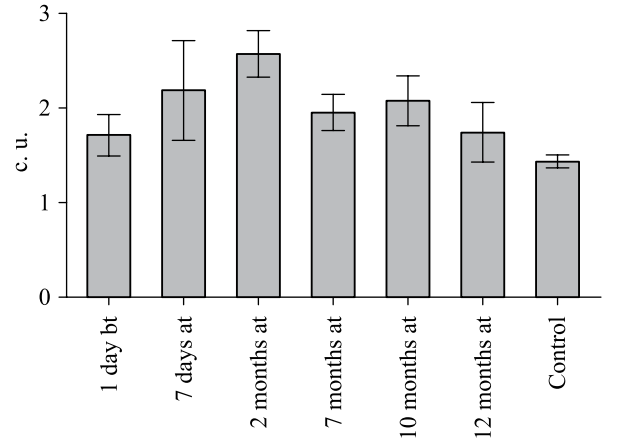


Fig. 5. The concentration of malone dialdehyde in erythrocytes in patients with kidney transplantation

on postoperative day 7–30, followed by restoration to normal values (Fig. 6).

In turn, analysis of catalase activity in RBCs showed that the activity decreased in kidney recipients during

the first month after surgery (p = 0.0040) (Fig. 7). No changes in catalase activity were observed in kidney donors (Fig. 8).

Analysis of results indicates that, during kidney transplant surgery, there is decreased RBC electrophoretic mobility, accompanied by increased aggregation and lipid peroxidation. In kidney donors, decreased RBC electrophoretic mobility is less pronounced and occurs against a background of increased serum MDA levels. Intensification of lipid peroxidation of cell membranes leads to compaction or destruction of the lipid bilayer, increased microviscosity, impaired functional activity of enzymes, changes in membrane permeability and surface charge, which can affect cell viability [22]. At the same time, it should be noted that decreased RBC negative charge reduces the suspension stability of blood and increases RBC aggregation, which slows down blood flow and ultimately leads to adverse changes in macrorheological blood parameters [23]. Since the studied groups had no increased catalase activity in response to increased oxidative stress, it can be assumed that the observed changes are mediated by the body's systemic response. Thus, activation of the sympathoadrenal system is accompanied by increased formation of reactive oxygen species during adrenaline autoxidation [24]. Interaction of adrenaline with RBC receptors activates phospholipases and increases lipid peroxidation. A breach in the structure of RBCs would lead to lower ability to effectively participate in tissue perfusion and oxygen delivery to cells.

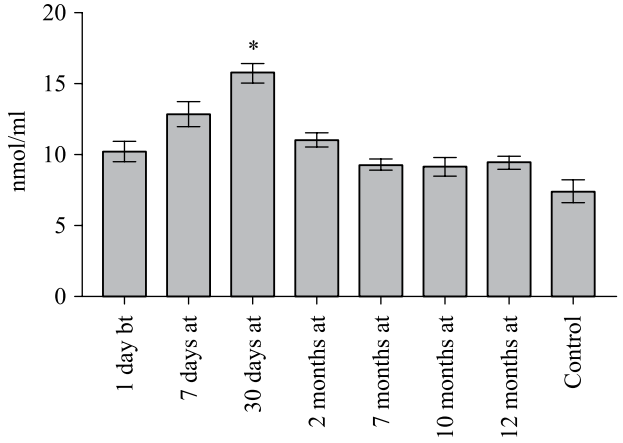


Fig. 6. The concentration of malone dialdehyde in erythrocytes of kidney donor

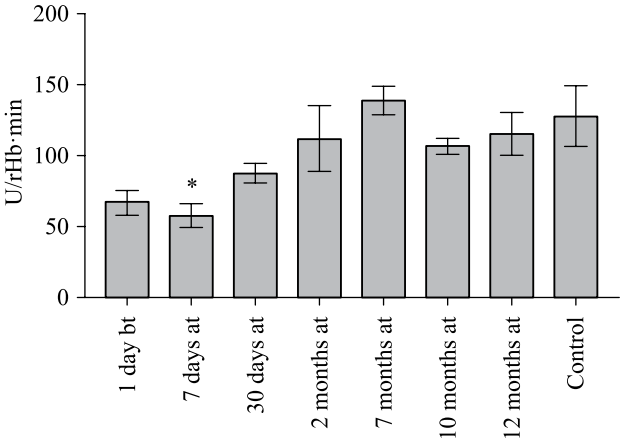


Fig. 7. The activity of catalase of erythrocytes in patients with kidney transplantation

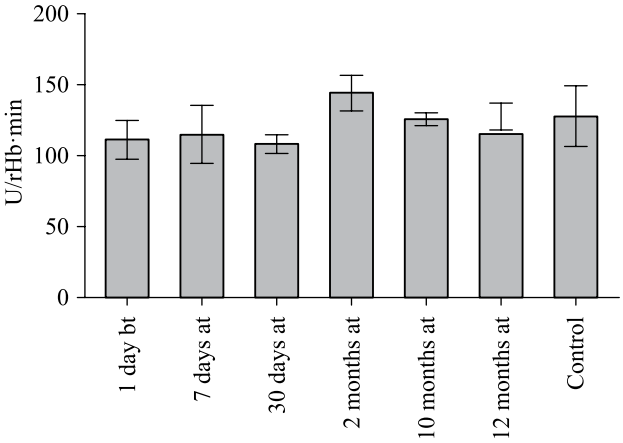


Fig. 8. The activity of catalase of erythrocytes of kidney donor

Ischemia leads to decreased energy metabolism due to depletion of macroergic phosphate reserves. Subsequently, perversion of specific intracellular metabolism, disturbances in enzymatic activity, intensification of anaerobic glycolysis, and pH changes are observed. Changes in enzymatic activity under hypoxia destabilize cell membranes and organelle membranes. This reduces membrane permeability, disrupts the functioning of ion pumps, and causes intracellular electrolyte disturbances. Normally functioning transplant cells reduce in number [15].

It is known that cell metabolism under ischemic conditions leads to lysis of cell membranes with the release of a large number of enzymes and vasoactive substances negatively affecting the recipient's state [25].

In turn, restoration of RBC electrophoretic mobility reflects decreased stress response and is observed with increased activity of the pituitary-adrenal system [26]. Cortisol plays a key role in maintaining homeostasis of the entire hypothalamic-pituitary-corticoid complex, which is responsible for development of non-specific mechanisms of the body's reactivity [27]. Corticosteroids cause an antioxidant effect [28], and it can be assumed that decreased serum MDA levels are mediated by increased corticosteroid concentration during development of adaptation processes.

So, this study has shown that kidney transplantation comes with some consequences both at the cellular and at the systemic level. At the cellular level, decreased stability of the membrane structure, which is largely dependent on lipid peroxidation processes, leads to reduced RBC electronegativity. At the systemic level, changes in

RBC electrophoretic mobility indicates a stress response with gradual increase in body resistance. Data obtained suggests there are changes in the functional properties of RBCs in kidney recipients and donors, which must be taken into account in therapeutic interventions.

*The authors declare no conflict of interest.*

## REFERENCES


1. *Sayegh MH, Carpenter CB*. Transplantation 50 years later – progress, challenges, and promises. *The New England Journal of Medicine.* 2004; 351 (26): 2761–2766. doi: 10.1056/NEJMon043418.
2. *Kiyokazu Akioka, Sirou Takahara, Seiji Ichikawa, Norio Yoshimura, Takahiro Akiyama, Shinichi Ohshima.* Factors predicting long-term graft survival after kidney transplantation: multicenter study in Japan. *World Journal of Surgery.* 2005; 29 (2): 249–256. doi: 10.1007/s00268-005-7531-8.
3. *Stolyar AG.* Effect of anemia on the results of kidney transplantation. *Clinical medicine (Russian Journal).* 2015; 93 (12): 24–27. [In Russ, English abstract].
4. *Roufosse C, Simmonds N, Clahsen-van Groningen M, Haas M, Henriksen KJ, Horsfield C et al.* Reference Guide to the Banff Classification of Renal Allograft Pathology. *Transplantation.* 2018; 102 (11): 1795–1814. doi: 10.1097/TP.0000000000002366.
5. *Campbell P.* Clinical relevance of human leukocyte antigen antibodies in liver, heart, lung and intestine transplantation. *Current opinion in organ transplantation.* 2013; 18 (4): 463–469. doi: 10.1097/MOT.0b013e3283636c71.
6. *Vanrenterghem Y.* Anaemia after renal transplantation. *Nephrology, dialysis, transplantation: official publication of the European Dialysis and Transplant Association – European Renal Association.* 2004; 19 (5): 54–58. doi: 10.1093/ndt/gfh1057.
7. *Molnar MZ, Czira M, Ambrus C, Szeifert L, Szentkiralyi A, Beko G et al.* Anemia is associated with mortality in kidney-transplanted patients. *Nephrology, dialysis, transplantation: official publication of the European Dialysis and Transplant Association – European Renal Association.* 2007; 7 (4): 818–824. doi: 10.1093/ndt/gfh1057.
8. *Vatazin AV, Zulkarnaev AB.* Kidney Transplantation as an Optimal Treatment for Chronic Kidney Disease. *The Journal of General Medicine.* 2013; 3: 47–52. [In Russ, English abstract].
9. *Robertson AJ, Nargund V, Grey DW, Morris PJ.* Low dose aspirin as prophylaxis against renal-vein thrombosis in renal-transplant recipients. *Nephrology, dialysis, transplantation: official publication of the European Dialysis and Transplant Association – European Renal Association.* 2000; 15 (11): 1865–1868. doi: 10.1093/ndt/15.11.1865.
10. *Gnjatic S, Wheeler C, Ebner M, Ritter E, Murray A, Altorki NK et al.* Seromic analysis of antibody responses in non-small cell lung cancer patients and healthy donors using conformational protein arrays. *Journal of Immunological Methods.* 2009; 1–2 (341): 50–58. doi: 10.1016/j.jim.2008.10.016. Epub 2008 Nov 28.
11. *Colvin MM, Cook JL, Chang P, Francis G, Hsu DT, Kiernan MS et al.* Antibody-Mediated Rejection in Cardiac Transplantation: Emerging Knowledge in Diagnosis and Management. *Circulation.* 2015; 18 (131): 1608–1639. doi: 10.1161/CIR.0000000000000093.
12. *An C, Akankwasa G, Liu J, Wang D, Cheng G, Zhang J et al.* Urine markers of renal tubular injury in idiopathic membranous nephropathy: A cross sectional study. *Clinica chimica acta; international journal of clinical chemistry.* 2019; 492: 7–11. doi: 10.1016/j.cca.2019.01.015.
13. *Belyakov NA, Semesko SG.* Antioxidant activity of human biological fluids: methodology and clinical value. *Efferentnaya terapiya.* 2005; 11 (1): 5–21. [In Russ, English abstract].
14. *Lobanova NA, Borovkov NN.* The disputable issues of influence of uraemic intoxication and free-radical processes on the development of anaemia of patients at terminal stage of chronic renal insufficiency. *Medical Almanac.* 2010; 3 (12): 152–155. [In Russ, English abstract].
15. *Vatazin AV, Zulkarnaev AB, Kantaria RO, Artemov DV.* Humoral pathogenesis ischemia/reperfusion injury factors in renal transplantation. *Ural'skij medicinskij zhurnal.* 2012; 13 (105): 94–97. [In Russ, English abstract].
16. *Romanov SV, Abaeva OP, Alexandrova OYu, Smirnova GYu.* Issues and perspectives of building a regional system of donor services (on the example of Nizhny Novgorod region). *Russian Journal of Transplantology and Artificial Organs*. 2019; 21 (1): 57–63. doi: 10.15825/1995-1191-2019-1-57-63. [In Russ, English abstract].
17. *Boyarinov GA, Yakovleva EI, Zaitsev RR, Bugrova ML, Boyarinova LV, Solov'eva OD et al.* Pharmacological Correction of Microcirculation in Rats Suffering from Traumatic Brain Injury. *Cell and Tissue Biology.* 2017; 11 (1): 65–72. doi: 10.1134/S1990519X17010023.
18. *Deriugina AV, Shumilova AV.* An influence of cytoflavin on oxidative stress and activity of Na/K-ATPase of erythrocytes after brain trauma. *Neuroscience and Behavioral Physiology.* 2017; 11: 51–55. doi: 10.17116/jnevro201711711151-55. [In Russ, English abstract].
19. *Kozinec GI, Popova OV, Budnik MI, Shmarov DA, Pogorelov VM, Procenko DD.* Elektricheskij zaryad kletok krovi. M.: Prakticheskaya medicina, 2007; 208.
20. *Krylov VN, Deryugina AV, Pleskova SN.* Electrophoretic mobility and morphometry of the rat erythrocytes at the stress effects. *Sovremennye tehnologii v medicine.* 2010; 4: 23–26. [In Russ, English abstract].
21. *Deryugina AV, Martusevich AA, Veselova TA.* Molecular and cellular mechanisms of stress response realization in the organism. *Izvestiya Ufimskogo nauchnogo centra Rossijskoj Akademii nauk.* 2015; 3: 58–63. [In Russ, English abstract].
22. *Alyasova AV, Terentiev IG, Tsybusov SN, Vedunova MV, Mishchenko TA, Shakhova KA et al.* Novel notions of the mechanisms of action of doxorubicin and ozone on malignant hepatic cells. *Sovremennye tehnologii v medicine* 2017; 9 (2): 145–149. doi: 10.17691/stm2017.9.2.18. [In Russ, English abstract].

23. *Sprague R, Ellsworth M, Stephenson A, Kleinhenz M.* Deformation-induced ATP release from red blood cells requires CFTR activity. *American Physiological Society.* 2010; 26 (2): 168–174.
24. *Bizenkova MN, Romantseva MG, Chesnokova NP.* Metabolic effects of antioxidants in acute anoxic hepoxia. *Fundamental research.* 2006; 1: 17–21. [In Russ, English abstract].
25. *Gol'din MM, Evseev AK, El'kov AN, Pinchuk AV, Tsar'kova TG.* Development and efficacy evaluation of the electrochemical predictor of complications in patients after kidney transplantation. *Transplantologiya. The Russian Journal of Transplantation.* 2015; 3: 6–10. [In Russ, English abstract].
26. *Antipenko EA, Derugina AV, Gustov AV.* An effect of cytoprotective therapy on stress resistance and compensatory abilities of patients with chronic cerebral ischemia. *S.S. Korsakov Journal of Neurology and Psychiatry.* 2015; 115 (12): 74–78. doi: 10.17116/jnevro201511511274-78. [In Russ, English abstract].
27. *Kalyi VV.* Synthesis and metabolism of glucocorticoid hormones in patients of young age groups having laryngeal cancer. *The Siberian Medical Journal.* 2010; 25 (1): 15–16. [In Russ, English abstract].
28. *Sergeev P.V.* Steroidnye gormony. M.: Nauka, 1984; 240.

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