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USE OF POLYCLONAL ANTIBODIES IN BRAIN-DEAD DONORS IN KIDNEY TRANSPLANTATION

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Objective. The objective of this study is to develop a therapeutic strategy for protecting grafts in order to improve the efficiency of kidney transplantation (KT) using polyclonal antibodies (pAbs) through elimination of activated forms of neutrophils, chemo- and cytokines from the donor's bloodstream, and a decrease in the level of expression of adhesion molecules on the renal vascular endothelium at the pre-transplant stage. **Materials and methods.** In 2017, we developed and for the first time applied a therapeutic strategy for ischemia-reperfusion injury (IRI) in a brain-dead donor (BDD). Given the limited time interval after brain death has been diagnosed, Timoglobulin (Sanofi Genzyme, France) was administered to the donor at a dose of 8 mg/kg intravenously for 6 hours. Before drug administration and immediately before the start of cold perfusion, a complete blood count and renal transplant biopsy were performed. The study group included 10 BDDs (mean age 39.3 ± 4.4 years) who received anti-thymocyte globulin (ATG). The comparison group included 10 BDDs (mean age 38.5 ± 4.3 years) who did not undergo the new strategy. Donor kidneys were transplanted to 40 recipients (average age 47.5 ± 4.3 years), who were also divided into 2 groups, depending on the graft received (with and without ATG). At the organ donation center, a biobank of specimens from donors of various categories, including those using the IRI therapeutic strategy and recipients for retrospective assessment of the effectiveness of pAbs, was formed. **Results.** Clinical blood test results show that in the ATG group, there was stable leukopenia (neutropenia and lymphopenia) of 1.46 ± 0.18x109/l. Fifteen (75%) recipients of kidneys obtained from donors with ATG had immediate graft function; in the control group – 10 (50%) recipients. **Conclusion.** Data obtained testify to the prospects of implementing the proposed strategy in clinical practice, which will improve the quality of the resulting grafts and their suitability for subsequent transplantation, prolong graft functioning due to elimination of leukocytes as a factor of IRI, prevention of early allograft nephropathy, increase in the donor pool by using expanded criteria donors (ECDs).

Keywords: organ donation and transplantation, ischemia-reperfusion injury, polyclonal antibodies.

INTRODUCTION

KT is a radical surgical intervention for end-stage renal disease. Therefore, it is generally accepted that KT is the treatment of choice and provides a better survival rate compared to long-term dialysis. It is also preferable in terms of quality and life expectancy of the recipient [1]. However, there is a worldwide shortage of suitable donor organs, and this method of treatment remains poorly available. In 2020, only 75,664 kidney transplants were performed worldwide [GODT. Global Observatory on Donation and Transplantation. 2020. http://www.transplant-observatory.org/reports. Accessed August 1, 2021].

For many years, the main source of donor organs was brain-dead donor (BDDs), whose organs were considered ideal. However, donor organ shortage has led to the use of transplants from expanded criteria donors (ECDs). This category of donors used to be regarded as an additional transplant resource, but today they are becoming the main resource [2]. The use of the ECD resource, which includes all donors >60 years old, or ≥50 years old, amidst comorbid conditions such as a history of hypertension, plasma creatinine levels >132 μmol/L just before the explantation procedure, diabetes mellitus, and excessive body weight (body mass index >30) can lead to a high risk of delayed function [3]. Nevertheless, this category of donors is considered an acceptable resource for KT [4].

Delayed graft function and poor long-term transplant outcomes remain a major obstacle to the expansion

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of the donor pool. KT from ECDs is characterized by more severe IRI. This is caused by hemodynamics instability in these kinds of donors. As a consequence, a complex set of events develops in such kidneys, which is characterized by more severe acute graft injury [5].

Delayed function occurs due to a pathophysiological complex of events associated with ischemic and hypoxic injury and reperfusion after hypothermic preservation, with a long recovery period after acute tubular necrosis [6]. Some researchers believe that in 23–38% of recipients of a kidney from a deceased donor, who receive hemodialysis within the first week after transplantation, the risk of graft loss increases to 40%. A significant proportion of kidneys obtained from donors over 50 years or from donors with high serum creatinine levels are not used [7].

Systemic inflammatory response, cell adhesion cascade and leukocyte activation are the key pathological mechanisms whose cumulative effect causes a sharp decrease in the functional reserve of the organs or, in the most unfavorable scenario, to irreversible changes leading to unsuitability for transplantation [8]. The most important in this case is the time of hemodynamic instability, warm ischemia, and "leukocyte mobilization" that occurs, having as a target the microcirculatory bed and endothelium of the organs. After triggering blood flow, activated neutrophils become the main source of free radicals and lysis enzymes production [9]. It should be noted that activation of adhesion molecules initiates neutrophil migration into the graft and leukocyte infiltration of tissues in general still at the donor stage, which further potentiates reperfusion kidney injury in the recipient body. So, IRI leads to increased length of hospital stay and reduced graft survival [10].

In turn, activated leukocytes play the leading role in the development of IRI. Mass adhesion of leukocytes to vessel walls and to each other eventually leads to the formation of large leukocyte conglomerates, which clog the vascular lumen and sharply impair venous outflow. In such clusters, individual leukocytes are rather strongly fixed to each other, but conglomerates themselves are of different sizes and sometimes weakly fixed to the vessel wall, so they are washed away by blood a few minutes after formation and carried away into larger vessels. However, during the terminal periods of tissue oxygen starvation, conglomerates persist, and according to Ivanov K.P. (1992), they stop "trains of red blood cell", leukocyte-platelet interactions occur, which leads to occlusion of vessels of increasing diameter and to their deformation. This subsequently explains the difficulty or impossibility of restoring microcirculation after deep hypoxia [11]. After restoration of blood flow, activated neutrophils producing free radicals and lysis enzymes become the main acting factor of IRI [12].

It has been established that at the time when the ischemia-reperfusion process begins, there is activation of neutrophils and further tissue injury through the release of reactive oxygen species (ROS), proteinases and cationic peptides [13]. Under the influence of proinflammatory cytokines and adhesion cascade, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase is activated, which causes production of large amounts of ROS by neutrophils [14]. Neutrophils block the capillary bed, preventing reperfusion, which leads to tissue necrosis and increased immune response [15]. Neutrophils secrete proinflammatory cytokines and chemokines, creating positive feedback [16]. In addition, neutrophil migration causes loss of epithelial barrier integrity and downregulates adhesion molecules [17].

IRI is initiated by an episode of ischemia, when blood supply to a part or whole organ is restricted, causing cell death, which is further exacerbated when blood flow resumes. Ischemia leads to tissue hypoxia, which causes accumulation of metabolites and ROS, namely superoxide, hydrogen peroxide, and hydroxyl radicals. ROS increase the amount of intracellular calcium, causing pH changes and simultaneously depleting adenosine triphosphate acid reserves, which leads to damage of cell organelles and cell necrosis. Prolonged ischemia time lasting from several minutes to half an hour causes irreversible effects, which are aggravated by reperfusion. During reperfusion, the ischemic tissue is filled with oxygen. This activates metabolites and ROS, which leads to an inflammatory response causing IRI [18].

According to Schofield Z.V. et al. (2013), IRI is a complex physiological process, but the undeniable leading role in it is played by neutrophils, which can penetrate the damaged tissue in just a few minutes after activation. Cytokines, ROS and the complement system are also important in the pathogenesis of IRI because they support, activate and enhance the destructive function of neutrophils. However, recent studies have again drawn attention to the role of neutrophils as a key player in the pathophysiology of IRI [12].

With the growing shortage of donor organs and increasing use of suboptimal quality organs, the study of pathophysiological processes developing in the body of a deceased donor has gained particular importance. Understanding the basis of pathological changes in grafts allows for consideration of new possibilities of influencing them, including through the use of new drug therapy techniques at the pre-transplantation stage in the donor's body. This can be an effective means of increasing the longevity of grafts obtained, including those from ECDs [19].

EpCAM (epithelial cell adhesion molecule) proteins are expressed on the membrane of renal epithelial cells, where it is involved in cellular and intercellular inter-

actions. The degree of EpCAM expression increases significantly during renal tissue repair and correlates with the severity of IRI effects. Transplant tissue regeneration requires a very high level of EpCAM expression for the development of cell proliferation, migration and differentiation processes [20]. It is assumed that prolonged and massive expression of EpCAM is associated with its tropism to cell proliferation and adhesion processes, which is observed after IRI. Thus, regulation of EpCAM expression is directly related to the need for renal tissue regeneration [21].

Research is ongoing around the world to find highly specific biomarkers of adverse conditions in transplantation. Creation of transplant biobanks, formed according to the standard methodology, should guarantee reliable statistical and clinical data. Evaluation of this data should result in the introduction of new ways of therapeutic correction of IRI in the donor's body. Collection, processing and storage of different types of samples at all stages of the donor process, during organ transplantation, as well as in the postoperative period, can serve as the basis for the creation of unique biobanks. Such biobanks will represent a matrix for end-to-end prospective longitudinal studies. The use of modern genomic techniques, including next-generation sequencing, will allow for a systematic approach to the study of ischemia-reperfusion and the genetic basis of transplant rejection response, will provide an individual approach to prescription of immunosuppressive drugs based on the genetic profiles of donor and recipient.

In the long term, it will be possible to address more ambitious tasks, such as studying the processes of "dying" with precise determination of the moment of the onset of irreversible changes. This will make it possible to revise the existing criteria for determining the suitability of organs for transplantation, moving away from an empirical approach to a genetically based one [22].

OBJECTIVE

To develop and implement a new method of drug therapy at the pretransplantation stage in BDDs, which can serve as an effective means of increasing the longevity of transplanted organs derived from ECDs, boosting the efficiency of KT through the use of polyclonal antibodies.

MATERIALS AND METHODS

A meeting of the Local Ethics Committee of St. Petersburg Research Institute of Emergency Medicine on March 15, 2017 approved the study "Use of polyclonal antibodies in brain-dead donors in kidney transplantation" and authorized its further development under the St. Petersburg Research Institute of Emergency Medicine. The present study was initiated in March 2017, with its five-year results evaluated in July 2022.

According to the official instructions for polyclonal antibodies thymoglobulin (Sanofi Genzyme, France), the patient develops deep lymphopenia (reduced lymphocyte count by more than 50% compared with the initial value) at day 1 after injection. A new method of therapeutic use of the drug, traditionally used only for the treatment of steroid-resistant rejection crises in recipients, seems to be an effective method of improving the quality of kidney transplants, by "turning off" the IRI leukocyte link, which, according to our hypothesis, will positively affect long-term transplant outcomes.

Given the existing time limitation for administration of the drug in the BDD (corresponding to the time interval required for the brain death diagnosis procedure in the donor), an empirical decision was made to increase the drug dose fourfold in order to achieve maximum reduction in the number of mobilized leukocytes. Thus, a proprietary technique for applying polyclonal antibodies in the BDD during KT was developed.

After the initial examination of the BDD by a team consisting of an anesthesiologist/resuscitator, a surgeon from the organ donation coordination center, and the transplant coordinator of the hospital, and a decision to start the brain death diagnosis procedure and plan kidney explantation, thymoglobulin, manufactured by Genzyme Polyclonals, S.A.S. (France), was administered to the donor within 6 hours at a 4-fold therapeutic dose. The drug dose was 8 mg/kg body weight. The drug was dissolved in 50.0 ml of saline and injected into the central venous catheter using a 0.14 ml/min single-syringe infusion pump.

Before the drug was injected and immediately before the start of cold perfusion, a complete blood count was performed. The study group included 10 BDDs (mean age, 39.3 ± 4.4 years) who received antithymocyte immunoglobulin (ATG); the comparison group consisted of 10 BDDs (mean age, 38.5 ± 4.3 years) without the new protocol; donor kidneys were transplanted to 20 recipients in the study group (mean age, 42.3 ± 3.1 years) and 20 recipients in the control group (mean age, 41.2 ± 3.3 years) depending on the graft received (with and without ATG). The general characteristics of the groups are given in Table 1 and Table 2.

In 2015, a transplant biobank was created for the first time in the Russian Federation under the St. Petersburg Donation Coordination Center. The biobank formed a collection of biological samples from donors and recipients, allowing to perform retrospective immunohistochemistry in kidney transplant biopsies to assess the effectiveness of the use of pAbs for IRI reduction in BDDs at the pre-explantation stage. Qualified specimen collection, processing, characterization and storage are performed for the functioning of the transplant biobank. Work with documentation and databases was standar-

Table 1

General characteristics of donor groups

Characteristics		Control group (no ATG), n = 10	Study group (with ATG), n = 10	P value
Age, years		38.5 ± 4.3	39.3 ± 4.4	0.08
Diagnosis	Stroke	4 (40%)	4 (40%)	
	Brain injury	2 (20%)	1 (10%)	
	Ruptured brain aneurysm	4 (40%)	6 (60%)	
Creatinine, μmol/l		66.5 ± 6.9	68.9 ± 7.3	
Urea, mmol/l		4.9 ± 0.5	5.6 ± 0.6	

Table 2

General characteristics of recipient groups

Characteristics		Control group (no ATG), n = 20	Study group (with ATG), n = 20	P value
Age, years		41.2 ± 3.3	42.3 ± 3.1	0.09
Diagnosis	Chronic glomerulo-nephritis	15 (75%)	15 (75%)	
	Autosomal dominant polycystic kidney disease	3 (15%)	2 (10%)	
	Stage 3 hypertension	2 (10%)	3 (15%)	

dized, algorithms for primary processing and storage of biosamples, and maintenance of proper sample condition were developed.

Incisional biopsies from kidney transplants served as the study material. Histological examination of kidney tissue was carried out at the National Center for Clinical Morphological Diagnostics, St. Petersburg, using the following methods:

1) Light microscopy was performed on paraffin sections using the following stains: H&E and PAS.
2) Immunohistochemistry performed by immunoperoxidase method using anti-EpCAM antibodies.

RESULTS

Immunohistochemistry in kidney transplant biopsies was performed to verify clinical data. In kidney transplant biopsies from BDDs in the comparison group, light microscopy demonstrated preserved histoarchitectonics of the renal parenchyma. The glomeruli had a single-loop capillary wall, with no signs of mesangial and endocapillary hypercellularity, no segmental glomerulosclerosis and crescents. There was artificial vacuolization of the epithelial cell cytoplasm of the proximal convoluted tubules. There were no signs of tubulointerstitial fibrosis. The walls of arterioles and small caliber arteries had no pathological changes. Immunohistochemistry study showed positive expression of EpCAM in the epithelial cell cytoplasm of the proximal convoluted tubules and no expression in other renal tissue structures (Fig. 1).

Histomorphological data indicating the resulting ischemic injury were assessed by the presence of positive expression of EpCAM molecules in the epithelial cytoplasm of the distal convoluted tubules. According to the study of kidney transplant biopsies from BDDs in the study group at the light microscopy level, the histological pattern remained the same. Immunohistochemistry, in turn, demonstrated a change in EpCAM expression – there was complete absence of EpCAM expression in all renal tissue structures, including the epithelial cytoplasm of the distal convoluted tubules.

Evaluation of clinical blood tests results in comparison groups: in the study group, there was stable leukopenia (neutropenia and lymphopenia) of $1.46 \pm 0.18 \times 10^9$/l (Fig. 2).

Also, according to laboratory data, a significantly higher level of Lipocalin-2 (neutrophil gelatinase-associated lipocalin, NGAL) was detected in the urine of BDDs in the control group compared to BDDs in the study group (Fig. 3).

The prognostic and diagnostic value of NGAL in acute kidney injury (AKI) is explained by the fact that it is excreted in the urine after renal parenchymal ischemia injury and is rightfully considered a "kidney troponin".

The high level of NGAL in BDDs in the control group demonstrates tubular lesions that precedes AKI.

Fifteen (75%) patients who got kidneys from donors who received ATG had immediate graft function, whereas there were 10 (50%) recipients in the group without the new protocol (Table 3, Fig. 4).

Five years after transplantation, serum creatinine and urea levels were lower in recipients of kidneys obtained from donors who received ATG (Table 3, Figs. 5 and 6).

Table 3

Assessment of kidney transplant function in two groups of recipients

Characteristics		Control group (no ATG), n = 20	Study group (with ATG), n = 20	P value
Graft function	Immediate	10 (50%)	15 (75%)	0.002
	Delayed	10 (50%)	5 (25%)	
Creatinine, μmol/L (after 5 years)		127.0 ± 6.8	101.0 ± 6.3	0.07
Urea, mmol/L (after 5 years)		8.1 ± 0.9	7.8 ± 1.1	0.08

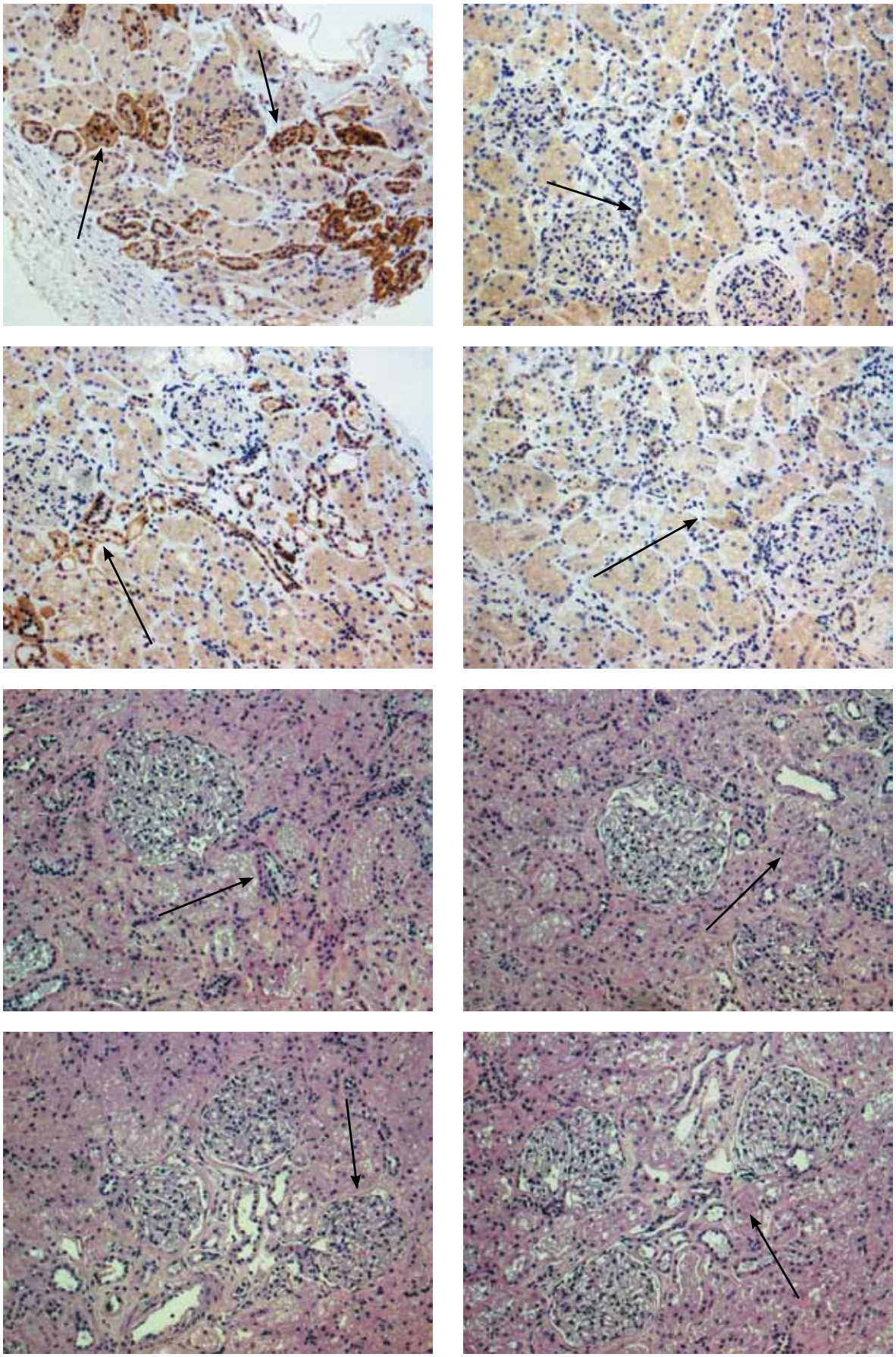

Fig. 1. Immunohistochemistry in null kidney graft biopsies from BDDs in the comparison group and the study group. In the comparison group there is a positive expression of EpCAM in the epithelial cytoplasm of the distal convoluted tubules – indicated by arrows in the figures in the left column. In the study group, there is no EpCAM expression in all renal tissue structures – indicated by arrows in the figures in the right column

Fifteen (75%) patients who received kidneys from donors injected with pAbs were observed to have immediate graft function, compared with 10 (50%) recipients in the comparison group. Five-year graft survival was 100% (n = 20) (pAbs), in contrast to 75% (n = 15). Serum creatinine levels 5 years after transplantation averaged 0.101 mmol/L in patients who received kidneys from BDDs injected with pAbs and 0.127 mmol/L in the comparison group.

Kaplan–Meier curves were plotted to assess the survival of kidney recipients and transplants in the study and comparison groups (Figs. 7 and 8).

ATG administered to donors can prevent IRI effects during KT by reducing the degree of necrosis and apoptosis and improving renal function, which is explained by a decrease in the expression of proinflammatory mediators.

In addition, given the functions and mechanisms of regulation of EpCAM expression, it can be concluded that our immunohistochemical study of kidney transplant biopsies demonstrate the effectiveness of pAbs in BDDs.

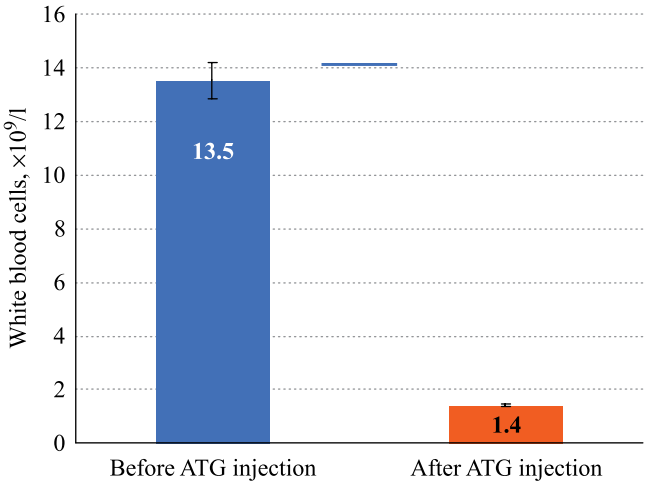

Fig. 2. WBC count in BDDs in the study groups before and 6 hours after ATG injection

DISCUSSION

A significant challenge in transplantation is the ability to use organs from ECDs without compromising immediate graft function and long-term graft survival.

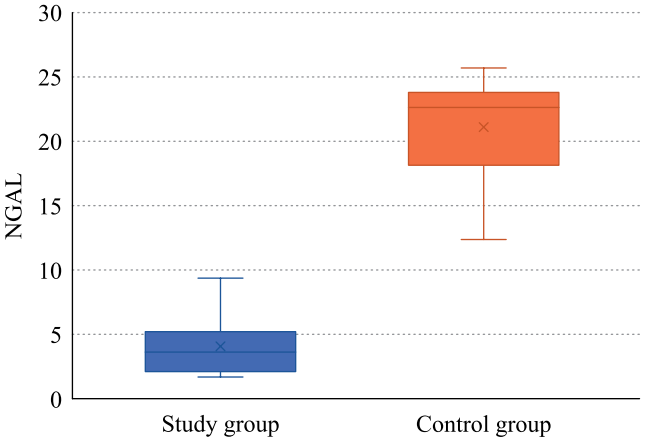

Fig. 3. Urinary NGAL levels in BDDs in the study and control groups

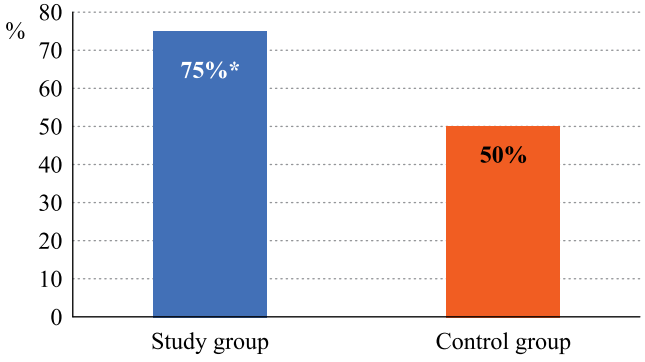

Fig. 4. Immediate function of kidney grafts in recipients in the study groups. * – p = 0.0008 compared to the control group

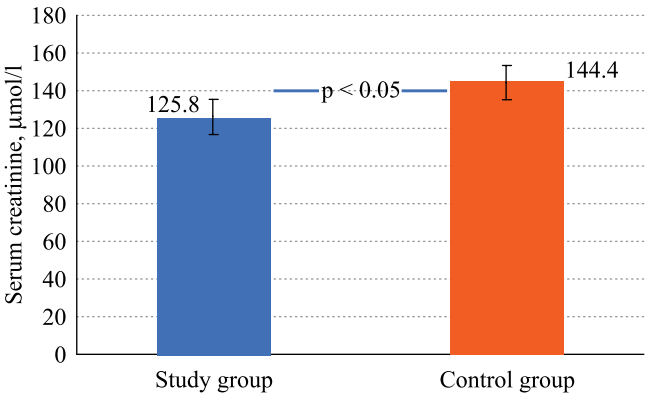

Fig. 5. Serum creatinine levels in recipients 5 years after kidney transplantation in the study groups

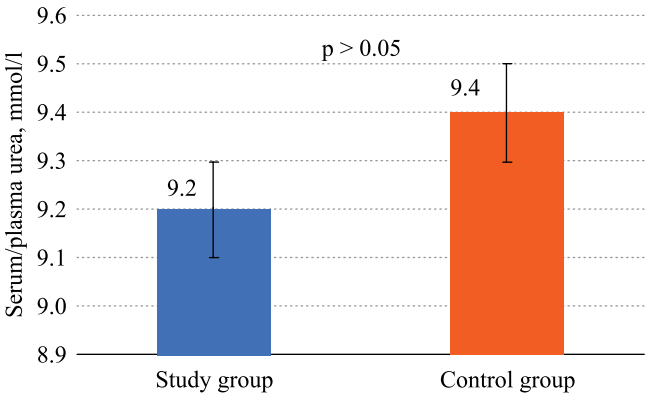

Fig. 6. Serum/plasma urea level in recipients 5 years after transplantation in the compared groups

Therefore, it is crucial to optimize each organ even before transplantation and to minimize additional damage to achieve the best possible function and avoid primary nonfunction, delayed function or acute rejection.

In the last decade, more information on the complex pathophysiology of IRI has emerged, opening the door for new therapeutic tools aimed at reducing the effects of IRI, tissue hypoxia as a result of microcirculatory bed blockage by recruited leukocytes. However, the existing methods for correcting IRI effects involve extracorporeal filtration and a whole arsenal of therapeutic options, which, however, still remain a compromise between desired effect and clinical reality. Despite significant progress in the study of the process underlying the mechanisms of renal graft dysfunction, treatment methods are still insufficient, and the results remain mixed.

For the first time in the clinical practice of KT, we proposed a new original scheme for IRI correction in BDDs. We propose a new way of protecting grafts by eliminating activated neutrophil forms from the donor's

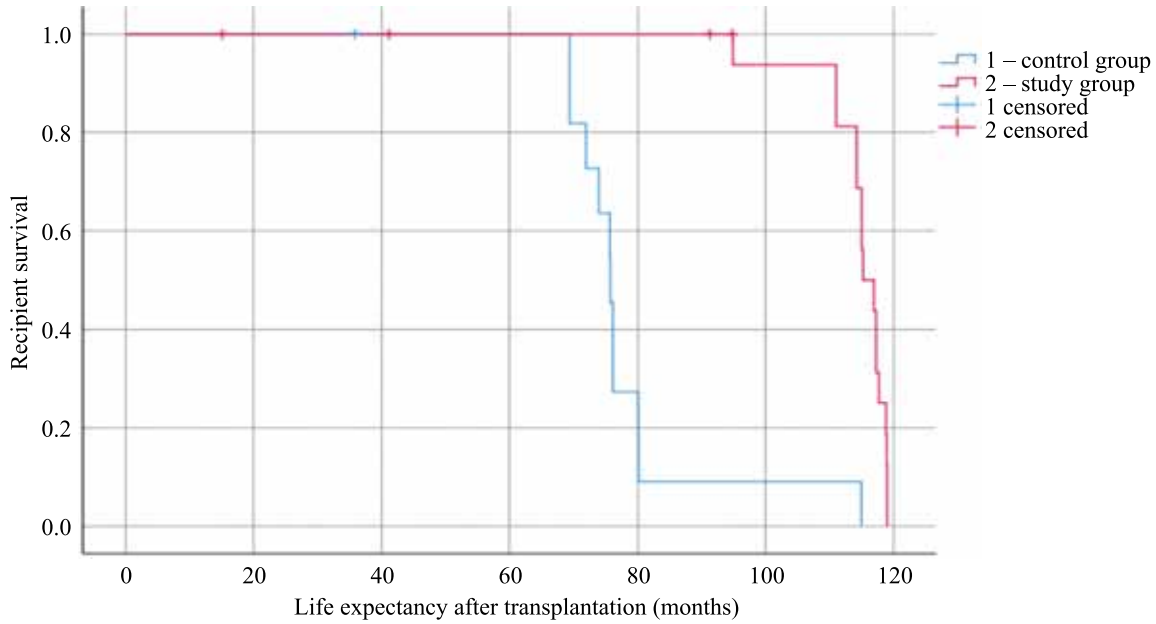

Fig. 7. Assessment of recipient survival

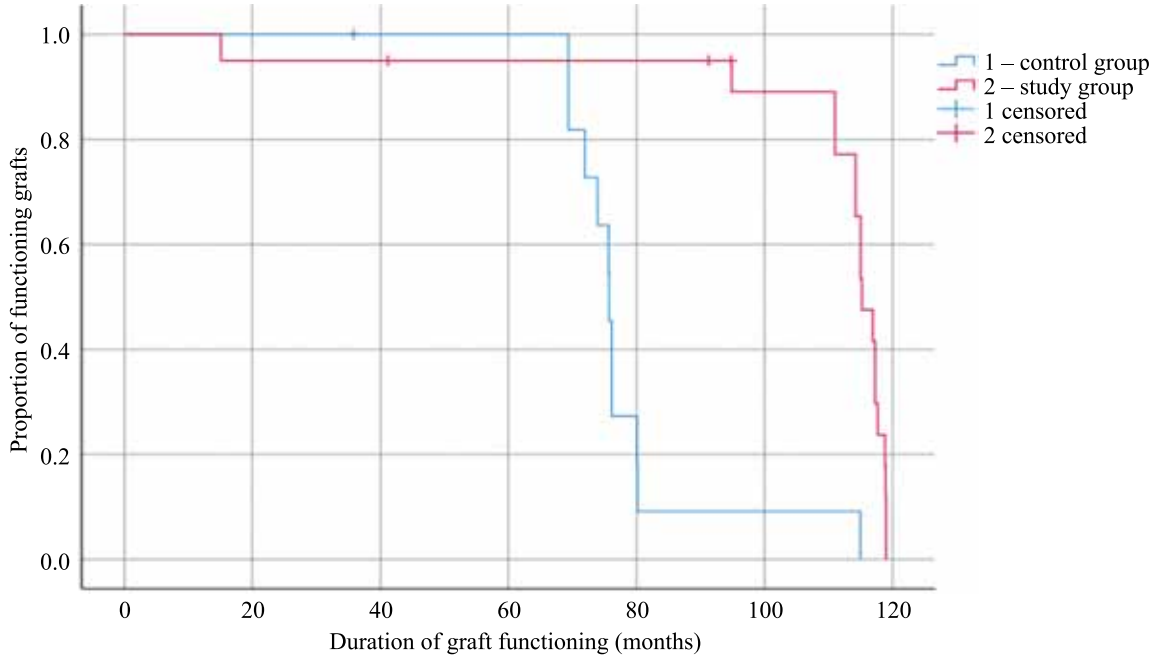

Fig. 8. Assessment of kidney graft survival

blood circulation, reducing the expression of adhesion molecules on the renal vascular endothelium by injecting pAbs into the BDD's body before explantation.

Thymoglobulin is a polyvalent drug, tropic to a variety of target antigens, which can be classified as immune response antigens, adhesion molecules and cellular transport. In this regard, therapeutic use of pAbs in BDDs, previously used only for the treatment of kidney recipients, seems to be an effective method of improving the quality of kidney transplants due to reduction of the IRI leukocyte link, which will positively affect long-term transplant outcomes. However, no reports on the use of pAbs in BDDs to improve the quality of kidney transplants have been found in available literature.

The developed protocol includes the following algorithm of actions when planning kidney explantation: after the BDD has been initially examined by a team consisting of the hospital's transplant coordinator, anesthesiologist/resuscitator and surgeon from the organ donation coordination center, negative serological test results for viral infectious agents has been obtained, and a decision to start a brain death diagnosis procedure has been taken, thymoglobulin was injected into the donor in a dose of 8 mg/kg body weight. Given the existing time limitation for administration of the drug in the BDD (corresponding to the time interval required for brain death diagnosis procedure in the donor), an empirical decision was made to increase the drug dose fourfold in order to achieve maximum reduction in the number of mobilized leukocytes.

Our hypothesis required not only clinical verification but also morphological confirmation, which is difficult to implement "here and now". It was important to preserve biospecimens from BDDs and recipients for future studies. For this purpose, a transplant biobank was created; it is a collection of biospecimens and related information in a form suitable for analysis.

The study results indicate the prospects of implementing the proposed strategy in clinical practice, which will improve the quality of resulting grafts and their suitability for subsequent transplantation, prolong graft functioning by preventing early transplant nephropathy, increase the donor pool by using ECDs, minimize the probability of acute rejection in recipients, reduce the length of hospital stay and, therefore, reduce economic costs. This protocol, using thymoglobulin, demonstrates the need to develop a similar domestic drug aimed at reducing leukocyte aggression in BDDs.

CONCLUSION

The study results indicate the prospects of implementing the proposed strategy in clinical practice, which will improve the quality of resulting grafts and their suitability for subsequent transplantation, prolong graft functioning by eliminating leukocytes as a factor of IRI and preventing early transplant nephropathy, and increase the donor pool by using ECDs. For retro- and prospective evaluation of transplanted organs, it is necessary to create biobanks at donation centers and transplantation departments.

The authors declare no conflict of interest.

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