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Глубокоуважаемые коллеги!

Среди реципиентов солидных органов определенное место занимают пациенты с так называемыми орфанными заболеваниями. Речь идет о редких заболеваниях, распространенность которых в популяции составляет не более 10 случаев на 100 тысяч населения. Это тяжелые прогрессирующие заболевания, приводящие к инвалидизации или смерти пациента, значимая часть которых обусловлена генетическими отклонениями либо аутоиммунными нарушениями. Характерные симптомы часто проявляются в детском возрасте, но почти в половине всех случаев болезнь может обнаруживаться и у взрослых.

Трансплантация органов выполняется при ряде орфанных заболеваний, у детей и взрослых, в случаях отсутствия или неэффективности этиотропной или патогенетической терапии. НМИЦ ТИО им. ак. В.И. Шумакова обладает опытом успешной трансплантации печени при синдроме Алажилля, гистиоцитозе, гликогенозе, дефиците альфа-1-антитрипсина, муковисцидозе (кистозном фиброзе), синдроме Уолкотта–Раллисона, синдроме Криглера–Найяра, синдроме Жубера, тирозинемии, наследственном гемохроматозе, галактоземии, первичной гипероксалурии 1-го типа, лейцинозе; трансплантации почки при атипичном гемолитико-уремическом синдроме, пароксизмальной ночной гемоглобинурии, первичной гипероксалурии, цистинозе; трансплантации легких при лимфангиолейомиоматозе легких, гистиоцитозе из клеток Лангерганса, не классифицированном

SOLID ORGAN TRANSPLANTATION IN THE TREATMENT OF ORPHAN DISEASES IN ADULTS AND CHILDREN

Dear respected colleagues,

Patients with the so-called orphan diseases occupy a certain place among solid organ recipients. By orphan diseases I mean rare diseases with a prevalence of no more than 10 people per 100,000 population. These are severe progressive diseases that lead to disability or death. Most of them are caused by genetic abnormalities or autoimmune disorders. Their characteristic symptoms often appear in childhood, but in almost half of all cases the disease can also be found in adults.

Organ transplantation is performed for a number of orphan diseases, in children and adults, in cases where there is no etiotropic or pathogenetic therapy or such is ineffective. The Shumakov National Medical Research Center of Transplantology and Artificial Organs has successfully performed liver transplantation for Alagille syndrome, histiocytosis, glycogenosis, alpha-1-antitrypsin deficiency, cystic fibrosis, Wolcott–Rallison syndrome, Crigler–Najjar syndrome, Joubert syndrome, tyrosinemia, hereditary hemochromatosis, galactosemia, primary hyperoxaluria type 1 and leucinosis. The center has also successfully conducted kidney transplantation for atypical hemolytic uremic syndrome, paroxysmal nocturnal hemoglobinuria, primary hyperoxaluria, and cystinosis. We have successfully carried out lung transplantation for pulmonary lymphangioleiomyomatosis, unclassified Langerhans cell histiocytosis, alpha-1 antitrypsin deficiency, cystic fibrosis, primary pulmonary hypertension, pulmonary arterial

в других рубриках, недостаточности альфа-1-антитрипсина, муковисцидозе (кистозном фиброзе), первичной легочной гипертензии, легочной артериальной гипертензии (ЛАГ, ассоциированная с врожденными пороками сердца, резидуальная ЛАГ и др.); трансплантации сердца при обструктивной гипертрофической кардиомиопатии, атаксии Фридрейха (аутосомно-рецессивная мозжечковая атаксия), врожденной мышечной дистрофии Эмери–Дрейфуса и др.

Более того, проведение трансплантации на ранних стадиях заболевания позволяет в большинстве случаев предупредить развитие метаболических, иммунных и других нарушений, приводящих к необратимой дисфункции жизненно важных органов и последующей инвалидизации. Своевременное проведение операции позволяет не только добиться выздоровления пациентов, но и устраняет имевшиеся проявления нарушений метаболизма.

При исследовании аутоиммунных и орфанных заболеваний мы разрабатываем комплексный подход, сочетающий решение фундаментальных задач и прикладные аспекты. Результаты исследований в области изучения и лечения орфанных заболеваний планируются к опубликованию в нашем журнале в 2023 году.

С уважением,
главный редактор
академик РАН С.В. Готье

hypertension (PAH associated with congenital heart disease, residual PAH, etc.). The Shumakov center has also recorded successes in heart transplantation for obstructive hypertrophic cardiomyopathy, Friedreich's ataxia (autosomal recessive cerebellar ataxia), Emery–Dreifuss muscular dystrophy, etc.

Moreover, transplantation in the early stages of the disease can, in most cases, prevent metabolic, immune and other disorders that lead to irreversible vital organ dysfunction and subsequent disability. With timely operation, the patient can fully recover and existing manifestations of metabolic disorders can be eliminated.

In the study of autoimmune and orphan diseases, we develop an integrated approach that combines fundamental problems and applied aspects. Results of our study and treatment of orphan diseases will be published in our journal in 2023.

Sincerely,

Sergey Gautier
Editor-in-Chief,
Member, Russian Academy of Sciences

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EXPERIENCE IN THE USE OF NEUTRALIZING MONOCLONAL ANTIBODIES IN KIDNEY TRANSPLANT RECIPIENTS WITH COVID-19

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Therapy with neutralizing monoclonal antibodies (mAbs) is particularly relevant during COVID-19 outbreaks in patients at high risk of severe disease, including kidney transplant recipients (KTRs). **Objective:** to evaluate the efficacy and safety of neutralizing mAbs in KTRs with mild to moderate COVID-19. **Materials and methods.** The retrospective study included 99 KTRs who received inpatient treatment for COVID-19 between September 1 and December 31, 2021. Patients were 52.0 ± 11.5 years old (M, 47.5%). Bamlanivimab/etesevimab combination drug at a dose of 700/1400 mg was used as mAbs. To evaluate the efficacy of mAbs therapy, two groups of patients were identified. Group 1 consisted of 33 KTRs who received mAbs as one of the therapy components, while group 2 consisted of 66 patients who received no mAbs. Discharge from the hospital or death was considered as the endpoint of follow-up. **Results.** In group 1, after the use of mAb, progression of pulmonary process was observed less frequently than in the control group with CT1-2 transformation to CT3-4 (9.1% vs. 30.3%, respectively, $p < 0.01$). Group 1 KTRs differed significantly from group 2 – lower need for ICU and ventilator care (6.1% vs. 27.3% and 3% vs. 19.8%, respectively). The groups were comparable by sex, age, body mass index, Charlson Comorbidity Index (CCI) and time after kidney transplant (KTx) at the onset of the disease and by ıaseline blood biochemistry parameter values at the time of hospitalization. Only C-reactive protein (CRP) and fibrinogen values were higher in the non-mAbs patients who were hospitalized later in the course of the disease (7.7 ± 3.2 days versus 4.6 ± 1.6 days in group 1, $p < 0.001$). The frequency of prescription of other therapies did not differ between the compared groups. Use of mAbs significantly reduced mortality from 19.7% in KTRs in group 2 to 3% in group 1 without adverse effect on graft function. **Conclusion.** The use of mAbs therapy in the early stages of COVID-19 in KTRs is safe, it prevents severe COVID-19, and reduces the incidence of adverse outcomes.

Keywords: kidney transplant recipients, COVID-19, neutralizing monoclonal antibodies.

The rapid spread of the novel coronavirus infection (COVID-19), which quickly reached pandemic proportions with severe consequences [1], prompted the international medical research community to conduct intensive research aimed at finding effective treatment approaches. Various options for therapeutic [2–6] and preventive measures against SARS-CoV-2 infection were developed and introduced into clinical practice within an unprecedentedly short period. Many of these measures, according to phase 3 clinical trials, have demonstrated high efficacy [7–10]. Given the clinical experience gained during the pandemic, which indicated frequent cases of severe disease, the therapeutic potential of neutralizing monoclonal antibodies (mAbs) – recombinant immunoglobulins derived from B cells of convalescent patients or humanized mice – is of great interest [11].

As early as November 2020, the first mAbs were approved by the US Food and Drug Administration for emergency use in patients with mild to moderate SARS-CoV-2 infection in the prehospital phase [12, 13]. To date, the number of mAbs approved for COVID-19 treatment and prevention is steadily rising, and more are under development or clinical trials [14]. The main mechanism of action of neutralizing mAbs of the IgG1 subtype is aimed at blockade of various epitopes of receptor-binding domain (RBD) of the spike (S) protein SARS-CoV-2, preventing its interaction with angiotensin-converting enzyme 2 (ACE2) on target cells and thus preventing

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virus penetration into them. For therapeutic purposes, mAbs are used both as monotherapy (regdanvimab, sotrovimab, etc.) and in combination forms (bamlanivimab/etesevimab, casirivimab/imdevimab, AZD8895/AZD1061, etc.).

Based on the experience with the use of mAbs in HIV patients, in which, similarly to COVID-19, there is a high frequency of virus mutations, it has been suggested that a combination of mAbs binding non-overlapping epitopes in the SARS-CoV-2 S protein reduces the likelihood of simultaneous failure of individual antibodies making such antibody cocktails. Thus, prophylactic and therapeutic use of casirivimab/imdevimab combination, tested on animals and confirmed in clinical practice, allows maintaining the neutralizing ability in many known mutations in the S protein, reducing the degree of viremia as well as the frequency and severity of pulmonary lesions compared to placebo [15–18]. In view of these data, the use of mAbs is particularly relevant for the treatment of patients at increased risk of severe disease with an adverse outcome, which include kidney transplant recipients [19–21].

The results of one of the first studies on the use of mAbs in 25 solid organ transplant recipients with COVID-19 showed that no deterioration was observed in any case and no inpatient treatment was required [22]. In another observation, after administration of sotrovimab, COVID-19 progression was observed in only 1 of 51 recipients; there were no deaths [23]. The use of bamlanivimab and casirivimab/imdevimab in patients after solid organ transplantation with mild to moderate SARS-CoV-2 infection also reduced the rate of hospitalizations compared to recipients who did not receive mAbs (15.3% vs 8.7%, respectively). However, the differences did not reach statistical significance. As in previous publications, no deaths were observed in the study group, unlike in the control group [24]. Taking into account the absence of similar studies in Russian practice to date, it is reasonable to evaluate the results of mAbs therapy in the Russian population of KTx recipients, which, thanks to the project (initiated by the Moscow Health Department) on the use of mAbs in the most vulnerable patient groups, has recently been introduced in the treatment regimens of SARS-CoV-2 infection in patients after KTx.

The aim of this study was to evaluate the efficacy and safety of mAbs for COVID-19 in KTRs.

MATERIALS AND METHODS

The retrospective study included 99 KTRs hospitalized for COVID-19 from September 1 to December 31, 2021 at the Department of Nephrology and Kidney Transplant Pathology, City Clinical Hospital No. 52, Moscow. The hospital was reassigned during the pandemic to provide medical care for COVID-19 patients. Patients' age was 52.0 ± 11.5 years (M – 47.5%), post KTx at the time of COVID-19 disease was 62.0 (28.0; 157.0) months. Distribution of patients according to the nature of the underlying disease that caused the end-stage chronic kidney disease (CKD) is shown in Table 1. SARS-CoV-2 infection was verified on the basis of RNA virus identification in nasopharyngeal and oropharyngeal smears by PCR and chest computed tomography (CT) data. Determination of IgM and IgG antibodies to new coronavirus antigens in the blood was an additional diagnostic method. Patients with severe COVID-19 (CT3–4) at the time of admission were not included in the study.

Table 1

Causes of end-stage CKD

Nosology	Number of patients	
	abs.	%
Chronic glomerulonephritis	48	48.5
Polycystic kidney disease	12	12.1
Systemic diseases	8	8.1
Diabetes mellitus (type 1/2)	7 (5/2)	7.1 (5.1/2.0)
Chronic pyelonephritis	6	6.1
Abnormal development of the urinary system	4	4.0
Kidney stones	3	3.0
High blood pressure	2	2.0
Thrombotic microangiopathy	1	1.0
Gout	1	1.0
Rheumatoid arthritis	1	1.0
Oncology	1	1.0
Nephropathy of unknown etiology	5	5.1
Total	99	100

In accordance with international guidelines [25] and Russian interim guidelines for the treatment of new coronavirus infection [26, 27], maintenance immunosuppression was modified in KTRs with a confirmed COVID-19: mycophenolic acid preparations were cancelled, calcineurin inhibitors were minimized, while increasing the prednisolone dose by 5–10 mg/day from the initial level. Target cyclosporine and tacrolimus levels were considered 30–50 ng/ml and 1.5–3 ng/ml, respectively.

COVID-19 complex therapy included antiviral drugs and anticoagulants.

In KTRs with early hospitalization ≤7 days from the onset of the disease, neutralizing mAbs (combination of bamlanivimab and etesevimab at a dose of 700/1400 mg) was added to the basic therapy. In cases of severe systemic inflammatory response, monoclonal antibodies to interleukin receptors (IL6, less often IL1β, IL17) or Janus kinase inhibitors and/or dexamethasone were used. Antibiotics, IV immunoglobulin, and plasma exchange/plasma infusions were administered as indicated.

To evaluate the effectiveness of mAbs therapy, two groups of patients were identified. Group 1 consisted of 33 KTRs who received mAbs as one of the first components of COVID-19 therapy, group 2 (control) included 66 patients for whom no antibody cocktail was used for

treatment. In the compared groups, such parameters as patients' age, time since KTx at the time of SARS-CoV-2 infection, time from COVID-19 onset to hospitalization, its duration, body mass index (BMI), CCI, character of lung lesion dynamics (according to CT data), initial laboratory indicators and frequency of other immunomodulatory therapy were evaluated. Renal graft function was determined by creatinine plasma levels.

The patient was discharged from the hospital or died at the end point of follow-up.

Statistical analysis

When distribution of continuous variables was normal, the mean values were calculated, and in cases of irregular distribution, the median was calculated. Comparative analysis of averages was performed using Student's t-test. Categorical variables were expressed as numbers or percentages and their differences were assessed by Pearson's χ^2 method. When comparing variables, differences were considered significant at $p < 0.05$. SPSS software package (version 22) was used for statistical data processing.

RESULTS

In 95 of 99 (96%) KTRs, COVID-19 was diagnosed by identifying SARS-CoV-2, and only in 4 patients was the diagnosis based on detection of IgM class of antibodies to the virus antigen with corresponding clinical and laboratory manifestations of the disease. All patients had a characteristic picture of viral pneumonia according to chest CT scans. The study and control groups were comparable by sex, age, BMI, CCI, and time after KTx by the onset of the disease (Table 2). However, KTRs in group 2 who were not treated with neutralizing antibodies were hospitalized later than group 1 patients.

Most of the biochemical blood parameters that were examined on admission to the hospital, including creatinine levels, indicating the state of transplanted kidney, did not differ in the compared groups. The exceptions were CRP and fibrinogen levels, which were significantly higher in group 2 patients (Table 3).

The course of the disease was more favorable in patients who received mAbs. They had a higher level of oxygen saturation and required oxygen support less frequently (Table 4). In this group, progression of pulmonary process with transformation of CT1–2 into CT3–4 was detected only in 9.1% of KTRs. In the group of patients who did not receive neutralizing antibodies, it was detected in almost one-third of cases ($p < 0.01$).

Mortality was 3% (1 of 33 KTRs) and 19.7% (13 of 66 patients) in group 1 and group 2, respectively, $p < 0.03$. The main cause of death was acute respiratory dis-

Table 2

Comparative characteristics of the two groups

Parameters	Group 1 (mAbs+), n = 33 (100%)	Group 2 (mAbs–), n = 66 (100%)	p
Gender (M), n	14 (42.4%)	38 (57.6%)	NS
Age, g; M ± SD	50.9 ± 10.9	52.5 ± 11.8	NS
BMI, M ± SD	25.5 ± 5.5	24.9 ± 5.9	NS
CCI, M ± SD	3.8 ± 1.7	4.2 ± 1.9	NS
Time since KTx, months, Me (25%; 75%).	43.0 (21.5; 120.5)	93.5 (36.8; 163.0)	NS
Duration of illness before hospitalization, days; M ± SD	4.6 ± 1.6	7.7 ± 3.2	0.001

Note: NS, no statistically significant difference ($p > 0.05$) between groups.

Table 3

Comparison of laboratory parameters in the analyzed groups

Parameters	Group 1 (mAbs+), n = 33	Group 2 (mAbs–), n = 66	p
Leukocytes, 10^9/L	5.9 ± 2.8	6.3 ± 2.9	NS
Lymphocytes, 10^9/L	1.0 ± 0.5	0.9 ± 0.5	NS
Platelets, 10^9/L	136.9 ± 44.4	129.5 ± 59.8	NS
Creatinine, μmol/L	162.0 ± 66.2	188.9 ± 86.4	NS
AST, IU/L	27.8 ± 12.4	29.9 ± 14.5	NS
ALT, IU/L	23.9 ± 15.7	20.9 ± 14.3	NS
LDH, IU/L	272.5 ± 102.4	274.6 ± 104.3	NS
Fibrinogen, g/L	5.0 ± 1.3	5.8 ± 1.6	0.01
D-dimer, ng/ml	269.5 (155.3; 470.0)	234.0 (129.0; 514.5)	NS
CRP, mg/L	18.2 (4.9; 46.5)	34.0 (10.4; 84.9)	0.02
Procalcitonin, ng/ml	0.2 (0.1; 0.4)	0.4 (0.3; 1.1)	NS

Note: NS, no statistically significant difference ($p > 0.05$) between groups.

tress syndrome (ARDS). At the same time, in group 1, ARDS developed in a patient with a history of severe complications, who had suffered acute humoral rejection less than a month before the onset of COVID-19. It was treated with anti-crisis therapy with plasmapheresis, immunoglobulin, and rituximab. In group 2, 6 of 12 KTRs had ARDS aggravated by pulmonary embolism (1 person), sepsis/multiple organ dysfunction syndrome (MODS) (2 persons), and hemorrhagic syndrome (3 persons); cardiac arrest was the cause of death in 1 case.

The study and control groups were comparable in terms of frequency of prescription of other immunobiological drugs (Table 5), which excluded the possibility of them having some influence on the outcome of mAbs treatment. Therapeutic plasma exchange (TPE) was used more frequently in patients who did not receive neutralizing antibodies.

Renal graft function in the compared groups did not differ both at the hospitalization stage and by the end of follow-up. Plasma creatinine levels decreased in all KTRs against the background of minimizing the dose of calcineurin inhibitors: in group 1 from 162.0 ± 66.2 μmol/L at hospital admission to 133.2 ± 46.0 μmol/L at the end of treatment ($p < 0.01$), and in group 2 from 188.9 ± 86.4 μmol/L to 151.1 ± 82.8 μmol/L, respectively ($p < 0.01$).

No serious adverse events were observed during therapy with neutralizing antibodies.

DISCUSSION

KTRs who receive continuous immunosuppressive therapy to maintain graft function are generally recognized to have a high rate of SARS-CoV-2 infection, a propensity for a more severe COVID-19 [23, 24, 29] and an inadequate response to vaccine prophylaxis even when using booster doses of vaccines [28–30]. The risk of COVID-19-associated death in this patient cohort doubles compared to patients without transplantation after adjustment for age, body mass index, and comorbidities [31]. In view of the above, drugs capable of inhibiting disease progression in its early stages, which include neutralizing mAbs, are considered a priority for the treatment of patients with a prognostically unfavorable outcome of the new coronavirus infection.

According to a number of studies, the use of mAbs in KTRs in the first 7 days of the disease reduces the viral load, the frequency of severe COVID-19 and, accordingly, the need for inpatient treatment [16, 32–35]. For instance, in a study by Wang A.X. et al. [36], mAbs therapy at the outpatient stage more than halved the need for hospitalization compared to the control group (14.5% vs. 30.8%, respectively). The authors compared the intensity of passive immunity after administration of bamlanivimab and casirivimab/imdevimab with the natural immunity that forms after COVID-19, based on quantitative analysis to assess the blocking activity of anti-SARS-CoV-2 class IgG. Almost all KTRs who received mAbs had a 90%–100% activity level for these antibodies early after administration and remained high for the next 3 months. In the comparison group, the vast majority of patients had low neutralizing antibody activity both early and in the long-term period after COVID-19 (less than 49%), which seems to be the reason for more frequent disease progression and the need for hospitalization in these patients [36].

In contrast to the above, in the present study, the results of mAbs were evaluated not in outpatients but in inpatients with KTRs. We, as well as other authors, con-

Table 4

Comparison of treatment outcomes in groups 1 and 2

Parameters	Group 1 (mAbs+), n = 33 (100%)	Group 2 (mAbs–), n = 66 (100%)	p
CT1–2 transformation to CT3–4	3 (9.1%)	20 (30.3%)	0.01
SpO_2, %; M ± SD	94.2 ± 6.0	87.1 ± 12.9	0.003
Need for oxygen support, n	6 (18.2%)	36 (54.5%)	0.001
Mechanical ventilation, n	1 (3%)	13 (19.8%)	0.025
Frequency of transfer to ICU, n	2 (6.1%)	18 (27.3%)	0.01
Length of hospital stay, n days; M ± SD	10.9 ± 6.1	14.8 ± 8.7	0.03

Table 5

Comparison of the frequency of prescription of immunomodulators and TPE

Therapy	Group 1 (mAbs+), n = 33 (100%)	Group 2 (mAbs–), n = 66 (100%)	p
IL-6 receptor blockers, n	30 (90.1%)	64 (97.0%)	NS
Dexamethasone, n	27 (81.8%)	55 (83.3%)	NS
Janus kinase inhibitors, n	20 (60.6%)	45 (68.2%)	NS
TPE, n	5 (15.2%)	27 (40.9%)	0.01

Note: NS, no statistically significant difference ($p > 0.05$) between groups.

firmed the positive effect of therapy with neutralizing antibodies against SARS-CoV-2 when administered early in the disease. In group 1 after bamlanivimab/etesevimab administration, an increase in the prevalence of pulmonary lesions, manifested by transformation of CTR1–2 into CTR3–4 (9.1% vs 30.3%, $p < 0.01$), was statistically significantly less frequent than in group 2 patients. KTRs in the study group were characterized by a higher level of oxygen saturation and correspondingly low need for oxygen support compared to the control group. As a consequence, group 1 less frequently required ICU treatment and ventilator use after mAbs administration than group 2 KTRs (6.1% vs. 27.3%, respectively, $p < 0.001$ and 3% vs. 19.8%, respectively, $p < 0.025$). At the same time, the compared groups were comparable by sex, age, BMI, CCI, and time since KTx by COVID-19 onset. They also did not differ in baseline blood biochemical parameters at the time of hospitalization. The exception was CRP and fibrinogen, which were higher in patients who received no mAbs therapy. This was most likely due to their later hospitalization from the onset of the first symptoms (7.7 ± 3.2 days versus 4.6 ± 1.6 days in Group 1, $p < 0.001$), which precluded the use of neutralizing antibodies in these KTRs in accordance with the selection criteria for treatment. This circumstance is an unconditional limitation in our study. Nevertheless, taking into account the comparability of the compared groups according to the main clinical parameters and the frequency of prescription of other immunomodulatory drugs, we believe that early use of antibody cocktails in KTRs with COVID-19 is effective. Therapy involving mAbs in these patients was associated with significantly lower mortality (3% in group 1 versus 19.7% in group 2). The findings are consistent with the results of a recently published meta-analysis that included 8 retrospective studies [37]. In a comparison of the mAbs+ ($n = 313$) and mAbs– ($n = 617$) patient groups, treatment with neutralizing antibodies was associated with both a reduced risk of severe disease (OR = 0.19, 95%CI: 0.08 to 0.42, $p < 0.0001$) and lower mortality from COVID-19 (OR = 0.16, 95%CI: 0.06 to 0.45, $p = 0.0005$).

CONCLUSION

Thus, neutralizing monoclonal antibody therapy administered early in COVID-19 demonstrates a favorable safety profile and high efficacy in KTRs. Early administration of mAbs prevents progression of pathological processes in the lungs, reducing the frequency of severe course and adverse outcomes. However, with accumulation of experience in the use of this group of drugs, the question about possible virus mutations against the background of treatment with neutralizing antibodies with the risk of disease recurrence is discussed more and more actively in the literature. The real effectiveness of reported prolonged action of some mAbs in preventing SARS-CoV-2 infection is also of interest. Answers to these and a number of other questions require further extensive research.

The authors declare no conflict of interest.

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ON INDICATIONS FOR REPEAT LIVER TRANSPLANTATION

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Objective: to study the causes of graft loss and indications for repeat liver transplantation (rLT). **Materials and methods.** We studied the experience garnered from 250 orthotopic full-size cadaveric liver transplantations in 228 patients from 1998 to 2021. The severity of the patient's condition at the time of intervention was estimated according to the MELD scale. Repeat surgeries were performed in 22 cases in 19 patients (analyzed group). **Results.** Organ preservation parameters, length of stay in intensive care unit (ICU), severity of postoperative complications in primary transplantations in general and in the analyzed group did not differ significantly. The main causes of graft loss were graft arterial insufficiency (57.9%) and hepatic artery thrombosis (21%). Severe early allograft dysfunction (EAD) and primary nonfunction accounted for 10.5%, portal vein thrombosis occurred in 5%, and chronic graft rejection was noted in 5% of cases. **Conclusion.** Arterial insufficiency is one of the leading causes of graft loss after liver transplantation. Early correction of arterial and biliary complications help in preserving graft viability.

Keywords: liver transplantation, liver graft loss, early arterial complications.

INTRODUCTION

Repeat liver transplantations (rLT) are performed in 10–15% of patients with severe graft dysfunction [1]. The most common reason for graft loss is arterial insufficiency of the graft. Acute hepatic artery thrombosis (HAT) without early correction leads to organ necrosis and development of hepatic and then multiple organ failure, sepsis [2]. Chronic ischemia is realized in the formation of multiple biliary strictures (ischemic cholangiopathy, IsC) with a gradual loss of organ function, requiring rLT in the long term [3]. Recurrence of viral, autoimmune hepatitis, hepatocellular carcinoma, chronic rejection, are much less common, and can be conservatively treated over a long period [4, 5]. Primary nonfunction (PNF) of the liver graft remains a separate problem, when only rLT can save the patient.

Objective. To study the possible causes of graft loss and indications for rLT.

MATERIALS AND METHODS

We studied the experience garnered from 250 orthotopic full-size cadaveric liver transplantations (OLT) in 228 patients from 1998 to 2021. There were 104 males and 124 females; their ages ranged from 18 to 64 years (mean, 44.9 ± 7.7 years). The severity of condition of the 228 recipients was assessed using the MELD scale; it averaged 17 ± 6.3 points at the time of intervention (before 2006, OLT were calculated retrospectively). Indications for OLT are presented in Table 1.

Repeat surgeries were performed in 19 patients (analyzed group), *three of them underwent retransplantation afterwards*. Indications for primary OLT were cirrhosis resulting from chronic viral hepatitis (CVH) C (8 cases, 42%), CVH B (3, 15.8%), mixed hepatitis (2, 10.5%), and primary biliary cholangitis (2, 10.5%) respectively. Budd–Chiari syndrome, primary sclerosing cholangitis, unspecified cirrhosis, and alveococcosis were the reasons for primary OLT in 4 recipients. The mean age of the subsequently re-transplanted recipients at the time of primary OLT (9 women and 10 men, 19 to 58 years) was 44.0 ± 10.7 years, MELD severity averaged 17.8 ± 4.8 points, and did not differ significantly between all 228 patients (17.8 ± 4.8 vs 17 ± 6.3 points, $P > 0.05$).

Table 1

Indications for OLT in all recipients

Etiology	n
Hepatitis C cirrhosis	65
Hepatitis B cirrhosis	28
Mixed hepatitis cirrhosis	7
Autoimmune liver disease	22
Cholestatic liver disease	36
Malignant liver tumors	23
Unverified cirrhosis	24
Parasitic liver diseases	2
Budd–Chiari syndrome	4
Wilson–Konovalov disease	2
Toxic hepatitis/cirrhosis	9
Transplant dysfunction	20
Others	8
Total	250

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Statistical comparison of parameters between the groups was performed using Student's t-test; Mann–Whitney U test was used to assess differences. Binary regression was used to identify risk factors of graft loss.

Cold perfusion during organ harvesting was performed with 8–16 liters of custodiol (HTK "Custodiol", Kohler, Germany) cooled to 2–4 °C. If steatosis was suspected, the suitability of the organ for transplantation was reassessed at the end of cold perfusion; if in doubt, an emergency morphological examination of the biopsy sample was performed in 37 cases (15%); *in 12 cases, the quality of the donor organ was unsuitable for transplantation. In our Center (Granov Russian Research Center for Radiology and Surgical Technologies), liver transplantation with more than 50% steatosis diagnosed during an emergency biopsy was practically not performed. Planned histological examination of biopsy specimens of the harvested organ in the main and study groups showed no significant difference.*

OLT stages. After hepatectomy, caval reconstruction was performed in 11 cases according to the piggyback technique, in 5 cases according to the classical technique (inferior vena cava (IVC) resection), and cavostomy in 3 cases. Temporary venous shunting was used in three recipients. Portal vein anastomosis was performed end-to-end. Given the high variability of the arterial anatomy of the hepatic artery, its reconstruction in orthotopic liver transplantation was represented by different variants.

Most often, reconstruction was performed by forming an anastomosis between the recipient's common hepatic artery (CHA) and the donor's native hepatic artery (NHA), 64.4% (n = 161). The second most common variant was anastomosis between the recipient's and donor's native arteries, 22.8% (n = 57). In other cases, peculiarities of arterial revascularization – 12.8% (n = 32) – were caused by the variants of the right hepatic artery (RHA) origin, more rarely by the presence of a significant accessory left hepatic artery (LHA). When the RHA of the donor organ branches off a separate trunk (from the superior mesenteric artery), the reconstruction was performed by forming an anastomosis between the donor RHA and recipient RHA. In this case, the donor CHA anastomosis was performed using both the LHA and recipient CHA. In some cases, an anastomosis between the RHA and the donor's gastroduodenal artery (GDA) was performed for RHA reconstruction. This anastomosis was formed at the "back table" stage. As a rule, the recipient LHA was used to anastomose the donor's accessory LHA, while the recipient RHA was used to anastomose with the donor's CHA or NHA. Most arterial anastomoses were formed with continuous sutures (the parachute technique was used) using Prolen 6/0 thread. A small number of anastomoses were formed with separate knotty sutures using Prolen 7/0–8/0 thread.

Biliary reconstruction using a T-tube was performed in 9 cases, internal drainage in 6, and hepatic enteroanastomosis in 2 patients. In the remaining cases, the ducts were not drained. After OLT, all patients received standard immunosuppression according to the triple scheme: cyclosporine/tacrolimus, mycophenolate/azathioprine, prednisolone. Basiliximab was administered for induction before graft initiation into systemic circulation and on day 4 after the operation. The duration of preservation stages, volume of replacement hemotransfusion, stages of surgery, and length of stay in the ICU were recorded. The degree of graft dysfunction was assessed according to the criteria by P. Salvalaggio et al. (2012) [6], and the severity of general surgical complications was assessed according to the Clavien–Dindo classification (2004) [7, 8].

Arterial insufficiency, established intraoperatively by Doppler flowmetry, was corrected by heparinization of the graft vascular bed, revision or reconstruction of the anastomosis zone, ligation of the gastroduodenal and/or splenic arteries. In the postoperative period, the state of hepatic arterial blood supply was assessed by Doppler ultrasound (DU) and spiral CT scan; correction was based on direct angiography results. Balloon plasty and stenting were used. In cases of biliary complications, we drained the bile ducts, resected them with the formation of hepatic enteroanastomosis or cholangiostomy, and removed necrotized liver tissue.

RESULTS

Repeat transplantations were performed in 19 (8.3%) out of 228 patients, among whom retransplantation was performed twice in three of them. The reasons for rLT were arterial insufficiency of the organ in 11 (57.9%) and HAT in 4 recipients (21%). Cases of early allograft dysfunction (EAD) and PNF were noted in 2 (10.5%), portal vein thrombosis (5%), and chronic rejection (5%) in 1 case each, respectively (Table 2).

The length of ICU stay and the severity of postoperative complications in 19 recipients who subsequently underwent rLT were comparable to all primary 288 OLTs.

Morphological evaluation of native donor liver biopsy specimens was performed in all patients of the analyzed group during primary OLT.

The degree of diagnosed macrovesicular graft steatosis, which was not a contraindication for transplantation, in the analyzed group was also not significantly different from all observations (incidence, 18% vs 16%, $P > 0.05$). In one case, 50% macrovesicular steatosis was not an obstacle to rLT, as the organ was used in a patient with PNF.

Causes of dysfunction varied in the small rLT group.

In the first case, severe EAD was manifested by persistent hyperbilirubinemia (>200 μmol/L) and lack of bile production. Immunosuppression failure resulted in graft rejection in the second recipient. Recurrent portal vein thrombosis in the third case was complicated by late multiple intrahepatic biliary strictures. In cases 4,

5, 6, 8, 11 and 13, the cause of ischemic cholangiopathy was arterial insufficiency of the graft in the late postoperative period. Extra-hepatic biliary-ductal necrosis was detected in 3 patients (No. 7, 16, 18), but there was no pronounced disturbance of arterial blood supply to the liver. Acute HAT with the development of necrotizing cholangitis and liver abscesses was diagnosed in three recipients (No. 9, 14, 15). Splenic artery steal syndrome in the late postoperative period caused intrahepatic biliary strictures in 10 cases. Arterial insufficiency with the formation of early biliary strictures was noted in the twelfth recipient, right portal vein thrombosis led to a sharp deterioration in his condition. Late HAT with the development of ischemic cholangiopathy was diagnosed in the seventeenth case. PNF, severe abdominal compartment syndrome was noted in the last patient. When comparing conservation parameters and intraoperative data, the following results were obtained (Table 3).

As follows from Table 2, median preservation parameters, intraoperative data for primary transplants in the main and analyzed groups did not differ significantly; regression analysis revealed no signs/risk factors of graft

Table 2

Causes of graft dysfunction, ICU length of stay and severity of postoperative period

Patient	Cause of graft dysfunction	ICU (days)	Clavien–Dindo
1. S.	Early graft dysfunction	20	III a
2. I.	Violation of immunosuppression regime, rejection	22	I
3. Z.	Portal vein thrombosis. Cholangiopathy	15	III b
4. M.	Arterial insufficiency. Necrosis of donor part of ducts	10	III a
5. A.	Common iliac artery occlusion. Ischemic cholangiopathy	9	III b
6. K.	Ischemic cholangiopathy	7	III a
7. S.	Necrosis of donor part of ducts	9	III a
8. V.	Critical vertebral artery stenosis. Ischemic cholangiopathy	4	II
9. K.	Early hepatic artery thrombosis. Necrosis of the donor part of ducts	14	III b
10. M.	Late steal syndrome, ischemic cholangiopathy	8	II
11. G.	Vertebral artery stenosis. Ischemic cholangiopathy	5	II
12. O.	Portal vein thrombosis. Arterial insufficiency. Ischemic cholangiopathy	15	III b
13. M.	Arterial insufficiency. Ischemic cholangiopathy	6	II
14. K.	Early hepatic artery thrombosis. Necrosis of the donor part of ducts	25	III b
15. S.	Early hepatic artery thrombosis. Necrosis of the donor part of ducts	13	III a
16. V.	Necrosis of the donor donor part of ducts	6	III b
17. S.	Late hepatic artery thrombosis. Ischemic cholangiopathy	5	III a
18. S.	Necrosis of the donor portion of the ducts	5	III b
19. K.	Primary nonfunction, severe compartment syndrome	7	III b

Table 3

Median graft preservation parameters and intraoperative data for primary transplantation in the main (n = 209) and analyzed (n = 19) groups

Parameters	OLT (n = 209)	OLT (n = 19)	95% CI, P
Cold ischemia (min) IQR	382.5 306–485	375 300–435	P > 0.05
Warm ischemia (min) IQR	45 35–55	50 45–55	P > 0.05
Liverless period (min) IQR	75 60–95	75 60–80	P > 0.05
Arterial revascularization (min) IQR	35 25–50	40 28.75–61.25	P > 0.05
Systolic BP at the start of arterial blood flow	*>100 mmHg – 62%* *<100 mmHg – 38%*	*>100 mmHg – 32%* *<100 mmHg – 68%*	*P > 0.05*
Replacement hemotransfusion (ml) IQR	1193 615–2099	972 412–2519	P > 0.05
Operation duration (min) IQR	445 430–490	515 500–545	P > 0.05
Initial hemoglobin level (g/l) *IQR*	*101* *96–108*	*95* *90–102*	*P > 0.05*

IQR, interquartile range.

loss in both groups. The indicators of systolic blood pressure at the start of arterial blood flow are noteworthy: in the study group, most of the recipients (68%) had blood pressure below 100 mm Hg.

Analyzing bilirubin, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels at day 0, 3 and 7 following OLT, it should be noted that there were higher values of cytolysis markers in the study group at day 0 and 3 after transplantation (Table 4); however, no significant difference was obtained (p > 0.05).

In the study group, 15 of 19 recipients with evident or latent arterial insufficiency of the graft developed biliary complications within 14 days to 37 months (mean 13.2 months). Before rLT, cholangiodrainage – percutaneous transhepatic drainage (n = 6) and endoscopic drainage (n = 1) – was performed in seven cases. Six patients required resection of necrotic ducts with the formation of an external cholangiostomy. Before rLT, the MELD score of the severity of patients' condition was 21.3 ± 4.6.

It should be noted that late HAT developed in 4 patients (not included in the analysis) after OLT. Regional thrombolysis, multilevel balloon angioplasty, recanalization attempts and arterial stenting were performed. All patients were included in the waiting list. However, the absence of a donor organ, a gradual increase in biliary and infectious complications led to sepsis and death in these patients. Inadequate arterial blood supply to the liver in the long-term period in the 228 OLT was observed in 32 cases, which accounted for 14% of all transplants. In 11 recipients with significant splenic artery steal syndrome, its embolization was performed, which led to a stable positive outcome in 8 cases. Two patients died from infectious complications, one from acute myocardial infarction. Successful correction of arterial insufficiency by balloon angioplasty and stenting was performed in 5 out of 8 cases of hepatic artery stenosis. Three recipients died of sepsis.

An example of an indication for repeat transplantation is a clinical observation where the fight against a series of arterial and biliary complications was a temporary success.

***Patient I.**, 57 years old. Her medical records show that cirrhotic transformation of the liver parenchyma was detected during laparoscopic cholecystectomy in 2014. In February and August 2021, endoscopic ligation was performed for bleeding esophageal varices. Considering the high risk of recurrent bleeding, the patient was hospitalized on September 06, 2021 at Granov Russian Research Center for Radiology and Surgical Technologies for the installation of a transjugular intrahepatic portosystemic shunt (TIPS). On September 8, 2021, the superior mesentericography was performed; celiacography. Conclusion: cirrhosis, portal hypertension, splenic vein thrombosis. Attempted TIPS installation was unsuccessful. Repeat TIPS surgery on September 13, 2021 was deemed technically impossible due to anatomical features.*

On September 18, 2021, the following surgeries were performed: laparotomy according to Dr. Starzl's technique, hepatectomy with preservation of the retrohepatic IVC, orthotopic piggy back liver transplantation, abdominal cavity drainage. Cold ischemia of the organ lasted for 4 hours 40 minutes, warm ischemia was 40 minutes.

Histological report No. 50622/2021 dated September 23, 2021: small nodular liver cirrhosis.

Doppler ultrasonography of the hepatic vessels, which was conducted on September 29, 2021, showed a significant decrease in the volumetric velocity of blood flow through the hepatic artery and increased inflow through the portal vein, signs of splenic artery steal syndrome. On September 29, 2021, superior mesentericography and celiacography were performed (Fig. 1).

The catheter was inserted into the distal part of the splenic artery trunk, six Cook steel coils with a coil dia-

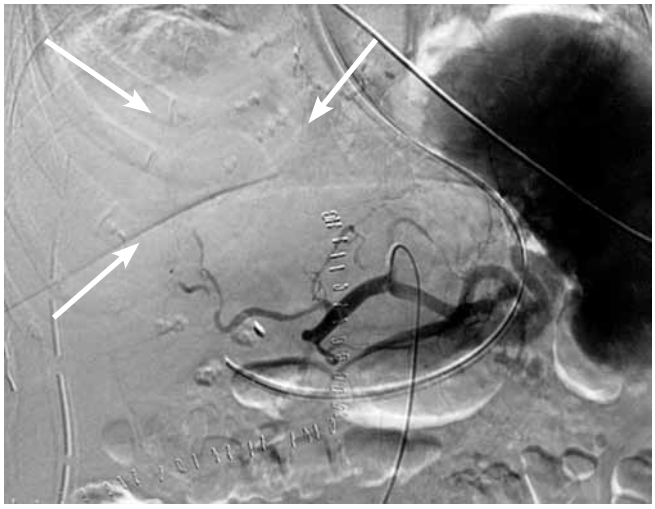

Fig. 1. Celiacography. Impoverishment of arterial architectonics at the segmental level is noted (arrows)

Table 4

Medians of bilirubin levels and cytolysis markers at day 0, 3 and 7 after OLT in primary transplantations in general and in the analyzed group

Indicators	Bilirubin (μmol/l)			ALT (IU/l)			AST (IU/l)		
Day after OLT	0	3	7	0	3	7	0	3	7
Main group (n = 209)	40.7	39.4	37.3	560	429	180	829	244	58
Study group (n = 19	41.7	44.4	36.7	822	585	158	1047	385	69

meter of 8 mm + hemostatic sponge were installed until blood flow was reduced (Fig. 2).

The splenic artery trunk was occluded with steel coils (black arrow). Arterial architectonics is traceable at the segmental level (white arrows).

DU of the hepatic vessels, which was conducted on September 30, 2021, shows a significant increase in the volumetric velocity of blood flow through the hepatic artery and decreased portal vein inflow.

Further control DU of the liver vessels conducted on October 26, 2021 (37 days after OLT) showed decreased linear velocity of blood flow in the native and right hepatic arteries in the absence of clinical and biochemical manifestations. Superior mesentericography and celiacography were performed, hemodynamically significant local narrowing of the native hepatic artery in the anastomotic area was visualized (Fig. 3).

A Medtronic Resolute 3.0 × 26 mm self-absorbable stent was placed coaxially to the stricture zone. Stenting was performed (Fig. 4).

According to the control DU of the hepatic vessels, which was conducted on October 27, 2021, there was a positive dynamics – acceleration of arterial blood flow in the NHA and the right branch of the hepatic artery. Free fluid between the left lobe of the liver and the stomach up to 300 ml was visualized. Ultrasound-guided puncture and catheter drainage of fluid accumulation was performed, 350 ml of the discharge with bile admixture was evacuated. The drainage yielded 400 ml of bile within a day. Magnetic resonance cholangiopancreatography conducted on October 28, 2021 visualized a defect in the common bile duct up to 5 mm with bile leakage into the abdominal cavity.

Relaparotomy, revision, irrigation of the abdominal organs, excision of the necrotic section of the common bile duct were performed. Hepatico-jejunal anastomosis was formed on a Roux-disconnected loop of small intestine, with Felker drainage of lobar bile ducts and Braun intestinal anastomosis.

Histological report #51548/2021 dated November 3, 2021: bile duct fragment with piecemeal necrosis, inflammatory infiltration.

Control fistulocholangiography that was conducted on November 08, 2021: adequate contrast enhancement of the bile ducts in both lobes of the liver. There are no leakages into the abdominal cavity.

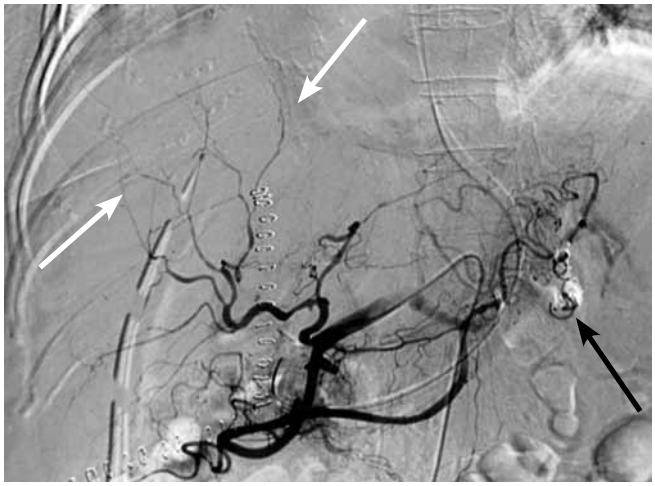

Fig. 2. Control celiacography after splenic artery embolization

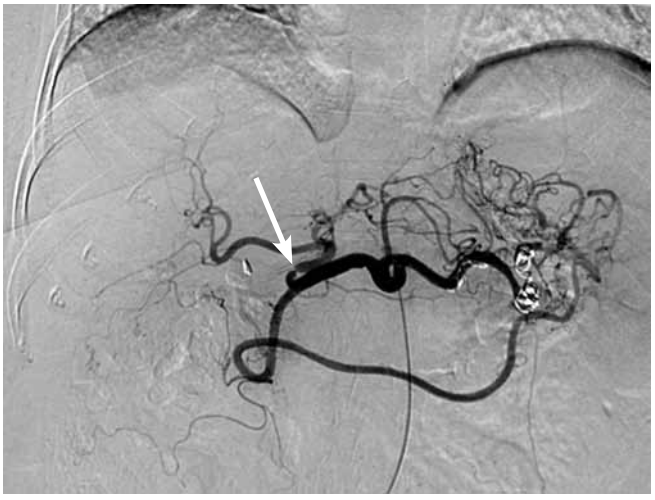

Fig. 3. Celiacography at day 37 after OLT. Hepatic artery stenosis (arrow) and impoverishment of the arterial pattern at the segmental level were revealed in comparison to the previous study

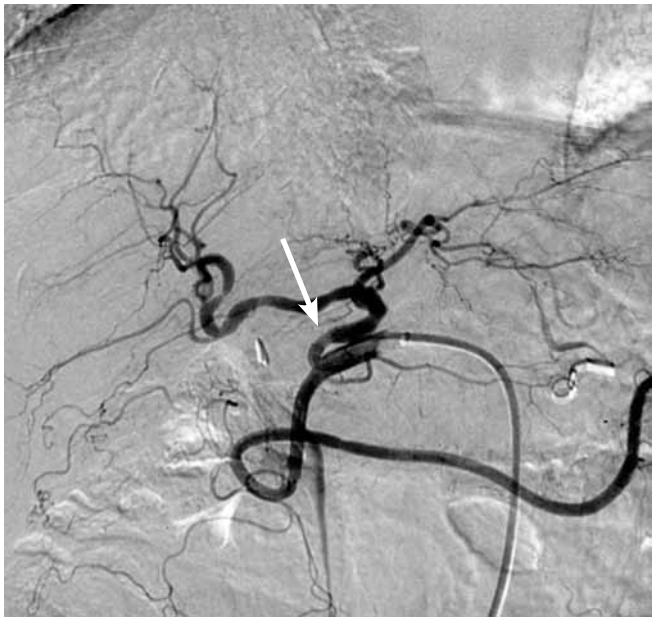

Fig. 4. Control hepatic arteriography. Hepatic artery stenting (arrow), stenosis eliminated, segmental arterial architectonics restored

DU that was conducted on November 12, 2021 showed there was a significant decrease in the volumetric blood flow through the hepatic artery.

Control celiacography and hepatic arteriography were performed. (November 12, 2021). GDA is found to be stealing hepatic blood flow, and there is decreased liver perfusion. In order to redistribute blood flow, the GDA was embolized – 4 Cook microcoils with a coil diameter of 6 mm were installed (Fig. 5).

Felker drains were removed on December 08, 2021. The patient was discharged on day 82 after OLT.

Blood tests at discharge: creatinine, 53.3 μmol/l; total bilirubin, 12.3 μmol/l; ALT, 7 units/l; AST, 12 units/l; albumin, 28 g/l. Tacrolimus concentration, 3.5 ng/ml. The patient had COVID-19 in February 2022 in a mild form with leukopenia 1.5–3.5 × 10^9/L, which required withdrawal of mycophenolic acid. In mid-March 2022, she noted jaundice. Liver DU conducted on March 29, 2022 showed no evidence of vascular complications in the graft, bile ducts were not dilated. Blood tests conducted on April 04, 2022: total bilirubin 470.4 μmol/L, ALT 1356 units/L, and AST 1172 units/L. Liver biopsy was carried out on April 5, 2022. Histological report #54845: liver tissue column with multiple piecemeal necrosis of hepatocytes. Diffuse lymphoplasmacytic infiltration of the stroma, most pronounced in the area of necrosis. Intracellular cholestasis. Protein degeneration of hepatocytes, areas of necrobiosis. Portal tracts were not revealed. Control celiacography, hepatic arteriography was performed (April 12, 2022). Decreased perfusion of the liver, distinct changes in the segmental arteries were noted (Fig. 6).

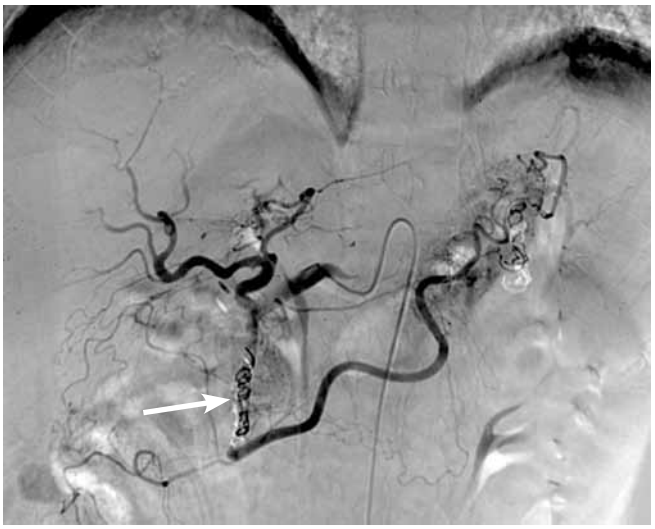

Fig. 5. Celiacography. Gastroduodenal artery occluded with metal coils (arrow). Obstruction of hepatic blood flow by the gastroduodenal artery was eliminated

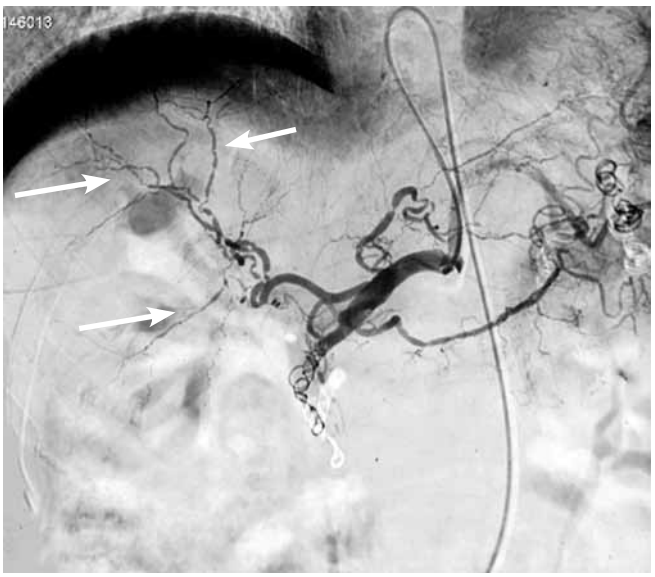

Fig. 6. Celiacography. Segmental arteries are clearly altered (arrows), with no pulse wave

In dynamics, there was an increase in hyperbilirubinemia up to 508 μmol/l (April 19, 2022). In order to assess liver function, the patient was tested with indocyanine green, plasma elimination rate was 2.6% per 1 min. Without repeat transplantation, further treatment seems to be unpromising, the patient was waitlisted for liver transplantation.

Currently, 9 (47%) out of 19 patients who underwent rLT are alive. The follow-up period ranged from 17 to 138 months. Graft function in the majority of recipients was considered satisfactory.

DISCUSSION

According to the results of the study, the proportion of recipients with rLT was 8.3%, which corresponds to the data of published works by national and foreign authors [9, 10]. It should be noted that over the past decade, the proportion of causes, such as relapses of viral, autoimmune hepatitis, rejections, resulting in the need for rLT, has decreased significantly. The rationale for this is the use of modern effective antiviral therapy drugs and immunosuppression [11–13]. In PNF, the lack of a donor organ remains an unsolved problem due to its shortage, imperfect allocation system and distribution between regions.

After 19 primary OLTs in patients who subsequently underwent repeat transplantation, PNF and severe EAD were observed in 2 cases. Relatively stable condition of the recipient and absence of severe infectious complications allowed to perform repeat transplantation after 8 and 21 days, respectively.

Analyzing all cases of severe graft dysfunction (13 of 228, 5.7%), its relatively low proportion can be explained by a small number of harvested organs from marginal donors, weighted interpretation of the result of emergency morphological examination of a harvested organ in case of doubt about its suitability. It should be noted active management of patients in the waiting list, aimed at improving the functional status in preparation for rLT,

as well as correction of post-transplant complications. In part, this provision can be confirmed by the fact that the severity of the condition of patients before rLT is comparable to that of the recipients before primary OLT; according to our data, MELD score was 21.3 ± 4.6 vs 17.8 ± 4.8 (P > 0.05).

In the majority of our cases (79%), the causes of graft loss were HAT (n = 4) and arterial insufficiency (n = 11). These problems were manifested in the formation of donor bile duct necrosis in the early postoperative period and multiple intrahepatic biliary strictures in the long term. Complications required repeat interventions – duct resection with external bile drainage, cholangiodrainage with numerous sessions of balloon angioplasty and stenting. Unfortunately, patients with late HAT (n = 4), significant hepatic artery stenosis (n = 3) and splenic artery steal syndrome (n = 2) that were not in the analyzed group died from infectious complications without waiting for repeat transplantation or effective endovascular correction [14].

CONCLUSION

Arterial insufficiency of the liver graft, which involves HAT, hepatic artery stenosis, and splenic artery steal syndrome, is a negative predictor of liver graft loss. To preserve the viability of the transplanted organ, diagnosing it as early as possible is extremely important. Despite the fact that adequate arterial blood supply can not always be achieved, it is necessary to use all available methods of surgical and endovascular correction of blood flow. Therefore, early diagnosis of arterial insufficiency is one of the key links in predicting graft dysfunction.

In this regard, active control and correction of arterial and biliary complications greatly contribute to liver function stabilization and, consequently, to graft preservation.

The authors declare no conflict of interest.

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ORIGINAL PANCREAS TRANSPLANT TECHNIQUE IN TERMS OF PREVENTION OF INTRA-ABDOMINAL PURULENT COMPLICATIONS

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A clinical case of pancreas transplantation (PTx) based on an original technique is presented. The applied technique made it possible to prevent the spread and involvement of the abdominal organs in an inflammatory process caused by postoperative graft pancreatitis, and to preserve the pancreas graft.

Keywords: pancreas transplantation.

INTRODUCTION

Pancreas transplantation (PTx) is one of the complicated sections of surgery due to the greater frequency and number of specific complications in comparison with transplantation of other solid organs [1–4]. Preservation of a fully functioning graft remains a pressing issue in this type of transplantation due to both post-transplant problems associated mainly with exocrine secretion and decreased microcirculation, and surgical complications, because they often lead to graft loss [5–7].

According to various literature sources, 30–40% of pancreas recipients develop surgical complications, and in the early postoperative period, graft loss occurs in 5–8% of cases because of intra-abdominal infectious complications, which are the result of duodenal anastomosis suture failure or graft pancreatitis [2, 4, 8]. This problem is caused by the fact that most transplantation centers still use the technique of intra-abdominal placement of the pancreas graft [9–12]. Infectious complications are the factors that aggravate the structure of the causes and increase post-transplant mortality rates [3, 13].

Occurrence of these problems in the postoperative period not only leads to unsatisfactory PTx outcomes, but also, to a certain extent, aggravates the issue of organ donor pool shortage.

To exclude intra-abdominal purulent complications, our clinic developed and put into practice an original PTx technique.

We present our own clinical observation of a patient in whom PTx was performed according to the original technique.

CLINICAL OBSERVATION

Patient B, female, 34 years old, with disability group 2, on July 17, 2018 presented with complaints of general weakness, increased blood pressure up to 240/115 mm Hg. Her past medical history shows that she has been suffering from type 1 diabetes mellitus for 2 years and 10 months. She has had kidney pathology for more than 4 years. Since February 13, 2018, she has been receiving renal replacement therapy three times a week. Since that time, she has been on the kidney transplant waiting list. In addition, she has proliferative diabetic retinopathy, myopia of the right eye, vitrectomy with vitreous tamponade of the left eye and secondary glaucoma.

On admission, his condition was moderately severe. Diuresis was up to 600 ml per day. There was an arteriovenous (AV) fistula on her left forearm (the last dialysis procedure was on July 17, 2018).

Preoperative diagnosis: type 1 diabetes mellitus, stage 5 chronic kidney disease (CKD), chronic renal failure (end stage), azotemia, stage 3 diabetic nephropathy, renal replacement therapy; long-term hemodialysis since February 13, 2018. Stage 2 Symptomatic (nephrogenic) hypertension, risk 3. FC 2 CHF. Stage 1 diabetic angiopathy of the lower extremities. Proliferative diabetic retinopathy, myopia of the right eye. Corneal leukoma in the left eye. Axonal sensorimotor distal mixed polyneuropathy of upper and lower extremities. Somatogenic asthenia.

The council of doctors decided to perform a surgical intervention on July 17, 2018, involving pancreaticoduodenal and kidney transplantation.

Description of the original technique of pancreas transplantation

At the first stage, the pre-transplant stage, the pancreas graft was prepared. The donor duodenum was sutured on both sides and arterial reconstruction of the splenic and superior mesenteric arteries was performed using a donor Y-shaped vascular insert (Fig. 1).

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In the recipient, the right retroperitoneal space was entered by a modified extraperitoneal Gibson access from the symphysis laterally and upwards parallel to the inguinal ligament [14], thus gaining access to the iliac vessels (Fig. 2).

The graft was placed in the right iliac fossa in an inverted position (with the head of the pancreas downward and the dorsal surface forward) along the wing of ilium. A venous anastomosis between the portal vein of the graft and the recipient's right iliac vein and an arterial anastomosis between the Y-shaped vascular insert and the recipient's right common iliac artery were formed according to the standard technique (Fig. 3).

Through a 6 cm peritoneal incision, we gained access to the small bowel, whose loop was removed from the abdominal cavity into the wound. Using the Roux technique, a 20 cm long section of the bowel was excluded from digestion; continuity of the gastrointestinal tract was restored by forming an end-to-side small-intestinal anastomosis, the free end of the bowel was sutured (Fig. 4).

At the next stage of the operation, we formed a side-to-side interintestinal anastomosis between the donor duodenum and the free end of the recipient's small bowel (Fig. 5).

At the final stage, the surgical intervention area was drained with two tubular silicone drains placed above and below the pancreas graft; the wound was sutured layer by layer (Fig. 6).

During the postoperative period, the patient underwent dynamic follow-up of the main laboratory indicators with a focus on plasma glucose and glycosylated hemoglobin levels.

Drug therapy in the postoperative period

Immunosuppressive therapy administered included: tacrolimus (1–24 days postoperatively) in an average daily dose of 4.69 mg (95% CI 4.27–5.11); mycophenolic acid (3–24 days postoperatively) in a dose of 720 mg per day. Along with this, the patient received methylprednisolone hormonal therapy (1–5 days postoperatively) at a mean daily dose of 123.75 mg (95% CI 89.74–157.76), then later (6–24 days postoperatively) at a dose of 16 mg per day.

Antibacterial therapy included a combination of carbapenems and synthetic antibacterials for 10 days – doripenem 1.5 g per day and metronidazole 15 mg per day, respectively, meropenem 2.0 g per day for 8 days.

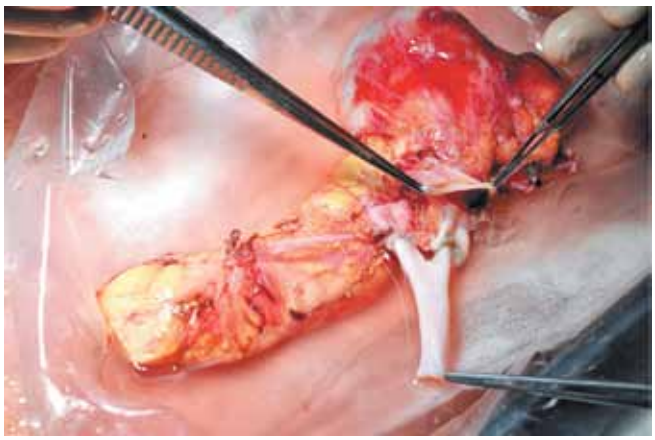

Fig. 1. Stage of pretransplant preparation of pancreas graft

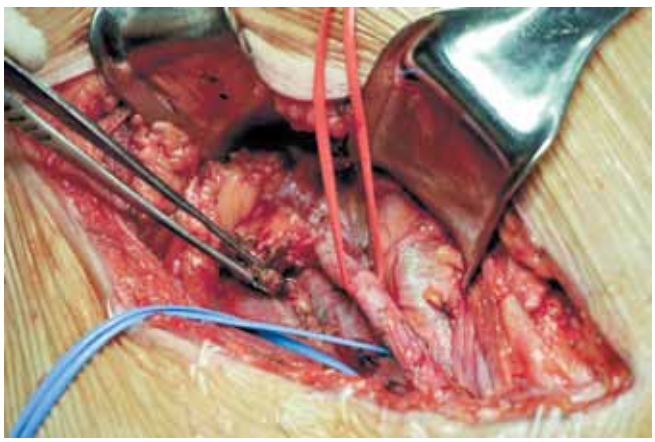

Fig. 2. View of access to the iliac arteries

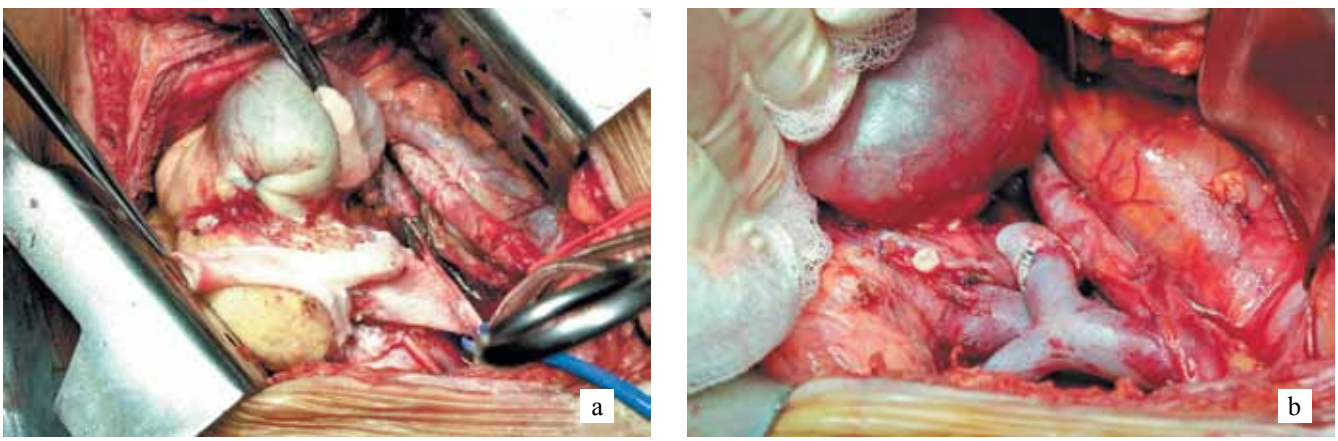

Fig. 3. Stage of vascular reconstruction of pancreas graft. a, formation of venous anastomosis; b, view of arterial anastomosis

Hypoglycemic episodes that occurred (Fig. 7) were corrected by insulin administration. The mean dose of insulin administered during the entire postoperative period was 4.66 units (95% CI 3.48–5.84).

RESULTS

In the first three days after surgery, during dynamic ultrasound examination of the pancreas graft, its longitudinal dimensions varied from 79 to 93 mm (86.0 ± 5.5 mm), transverse dimensions varied from 29 to 34 cm (31.2 ± 2.5 mm). Parenchymal echogenicity remained normal; echo pattern was homogeneous. Blood flow velocities in the vascular anastomosis zone and in the artery were within admissible values. Venous anastomosis: V_{max}, 105.7 ± 14.4 cm/s; arterial anastomosis: V_{max}, 148.3 ± 25.8 cm/s; Ri, 0.77 ± 0.06. Pancreas artery 1: V_{max}, 67.3 ± 15.2 cm/s; Ri, 0.70 ± 0.04; pancreas artery 2: V_{max}, 57.3 ± 9.8 cm/s; Ri, 0.75 ± 0.11. Visualized sizes of fluid accumulations along the graft bed ranged from 14 to 37 mm (27.0 ± 8.4 mm), in the transverse direction, from 6 to 14 mm (12.3 ± 6.8 mm). The daily volume of serous hemorrhagic exudate discharged through the drains installed in the graft bed did not exceed 220 ml (170.0 ± 55.7 ml).

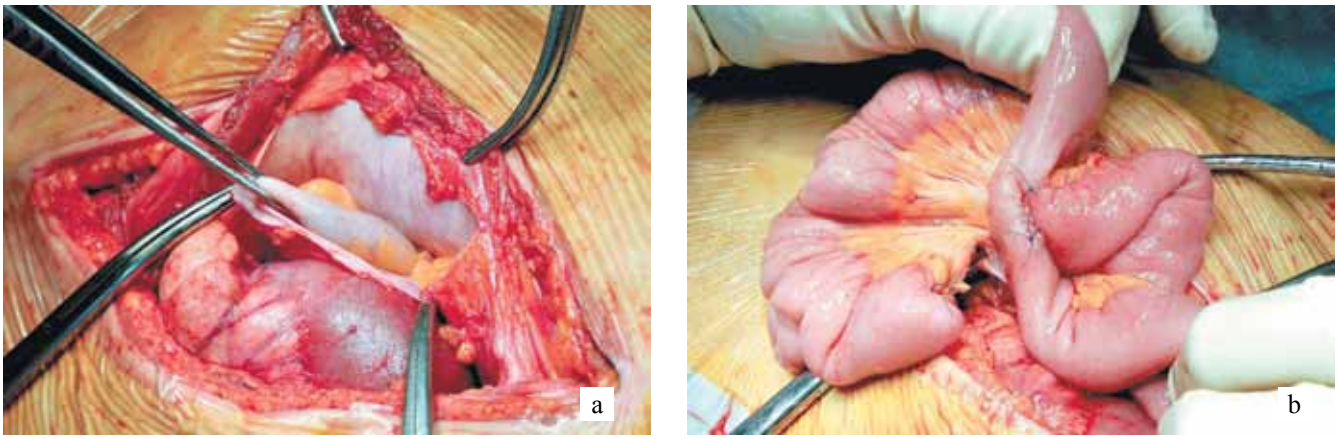

Fig. 4. The stage of preparation of the small bowel loop. a, peritoneum opening; b, view of the loop disconnected from digestion by Roux-en-Y bypass

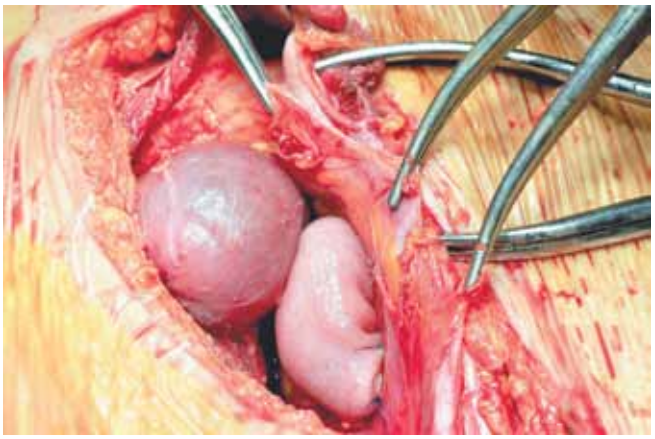

Fig. 5. View of formed anastomosis between recipient's small bowel loop and donor's duodenum

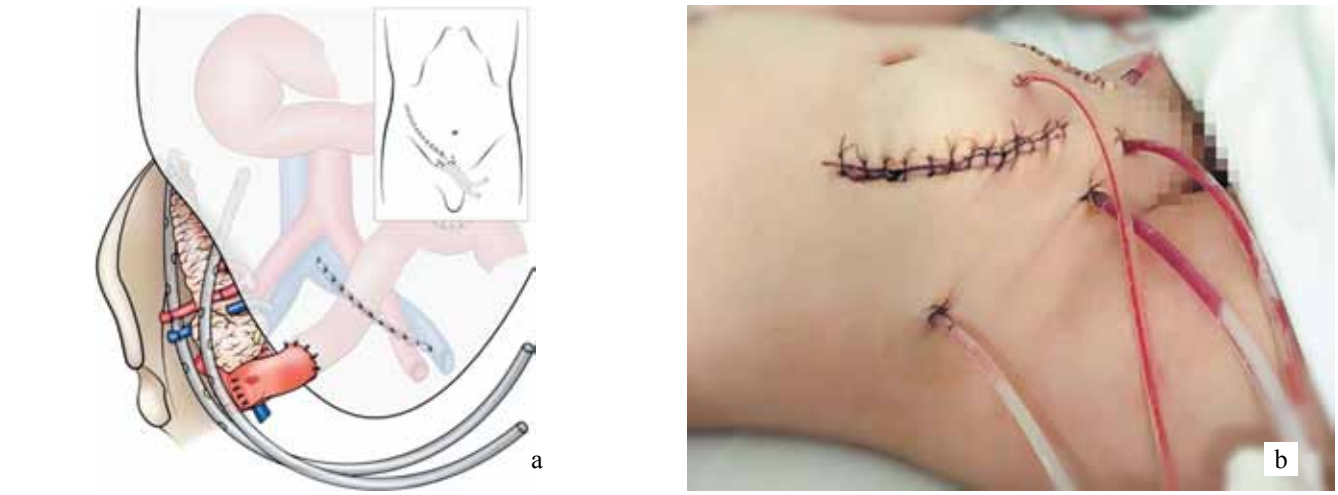

Fig. 6. General view of the operation. a, schematic representation of pancreas transplantation according to the original technique; on the right: drainage of the intervention area; b, drainage of the surgical intervention area

From day 4 to day 7 after the operation, ultrasound examination of the pancreas graft showed that its longitudinal dimensions varied from 80 to 89 mm (85.0 ± 2.0 mm), transverse dimensions varied from 34 to 40 cm (36.8 ± 2.0 mm). Parenchymal echogenicity was medium, echo pattern was heterogeneous. Blood flow velocities in the vascular anastomosis zone and in the artery had the following characteristics. Venous anastomosis: V_{max} – 95.3 ± 20.6 cm/s; arterial anastomosis: V_{max}, 132.5 ± 20.2; Ri, 0.72 ± 0.03. Pancreas artery 1: V_{max}, 70.5 ± 13.4 cm/s; Ri, 0.69 ± 0.02; pancreas artery 2: V_{max}, 69.8 ± 12.2 cm/s; Ri, 0.65 ± 0.04. The size of fluid accumulations along the graft bed ranged from 28 to 49 mm (37.1 ± 7.5 mm), in the transverse direction, from 12 to 37 mm (22.9 ± 11.8 mm). It should be said that during this period, the volume of exudate flowing daily from the graft bed through the drains increased by almost one and a half times – 383.6 ± 47.6 ml – and its character changed to purulent.

Ultrasound examination of the pancreas graft showed that from day 18 to day 23, the echogenicity of the gland parenchyma returned to normal, echo pattern became homogeneous. Longitudinal dimensions of the graft ranged from 72 to 80 mm (80.4 ± 5.1 mm), transverse dimensions ranged from 22 to 40 cm (33.3 ± 5.9 mm). Blood flow velocities in the vascular anastomosis zone and in the artery had the following characteristics. Venous anastomosis: V_{max}, 118.6 ± 15.2 cm/s; arterial anastomosis: V_{max}, 107.0 ± 1.7; Ri, 0.82 ± 0.05. Pancreas artery 1: V_{max}, 60.5 ± 2.0 cm/s; Ri, 0.65 ± 0.09; pancreas artery 2: V_{max}, 53.6 ± 8.2 cm/s; Ri, 0.72 ± 0.06. The size of fluid accumulations along the graft bed varied from 0 to 9 mm (3.4 ± 2.6 mm), in transverse direction – from 0 to 5 mm (1.8 ± 0.4 mm). It should be noted that in this time interval, the daily volume of exudate flowing through the drains significantly decreased (20.3 ± 1.7 ml), and its volume through the abdominal cavity drain did not exceed 50 ml of serous discharge. By day 24, the drains were completely removed.

Before the patient was discharged from the hospital (day 24 after surgery), she underwent ultrasound scan of the graft and abdominal CT angiography scan.

Triplex ultrasound scan protocol. Position of the pancreas graft in the iliac area on the right side. Size: 72 × 22 mm. Echogenicity is normal, echo pattern is homogeneous. Venous anastomosis: in the anastomosis area V_{max}, 100 cm/s; distally, 24 cm/s. Arterial anastomosis: with external iliac artery V_{max}, 90/22; Ri, 0.75. Graft artery 1: V_{max}, 69/14 cm/s; Ri, 0.79; graft artery 2: V_{max}, 48/9 cm/s; Ri, 0.8.

Abdominal CT scan protocol. Abdominal and retroperitoneal CT scan with bolus contrasting revealed: condition after kidney transplantation, pancreas transplantation. The renal graft is visualized in the left iliac region, measuring 103 × 53 × 75 mm. The parenchyma is uniformly contrasted. The artery supplying the graft is not narrowed. The excretory function of the kidney was preserved. In the right iliac region, the pancreatic graft is visualized, measuring 32 × 16 × 52 mm. The duct of Wirsung is up to 3.5 mm in diameter. The arterial vessel

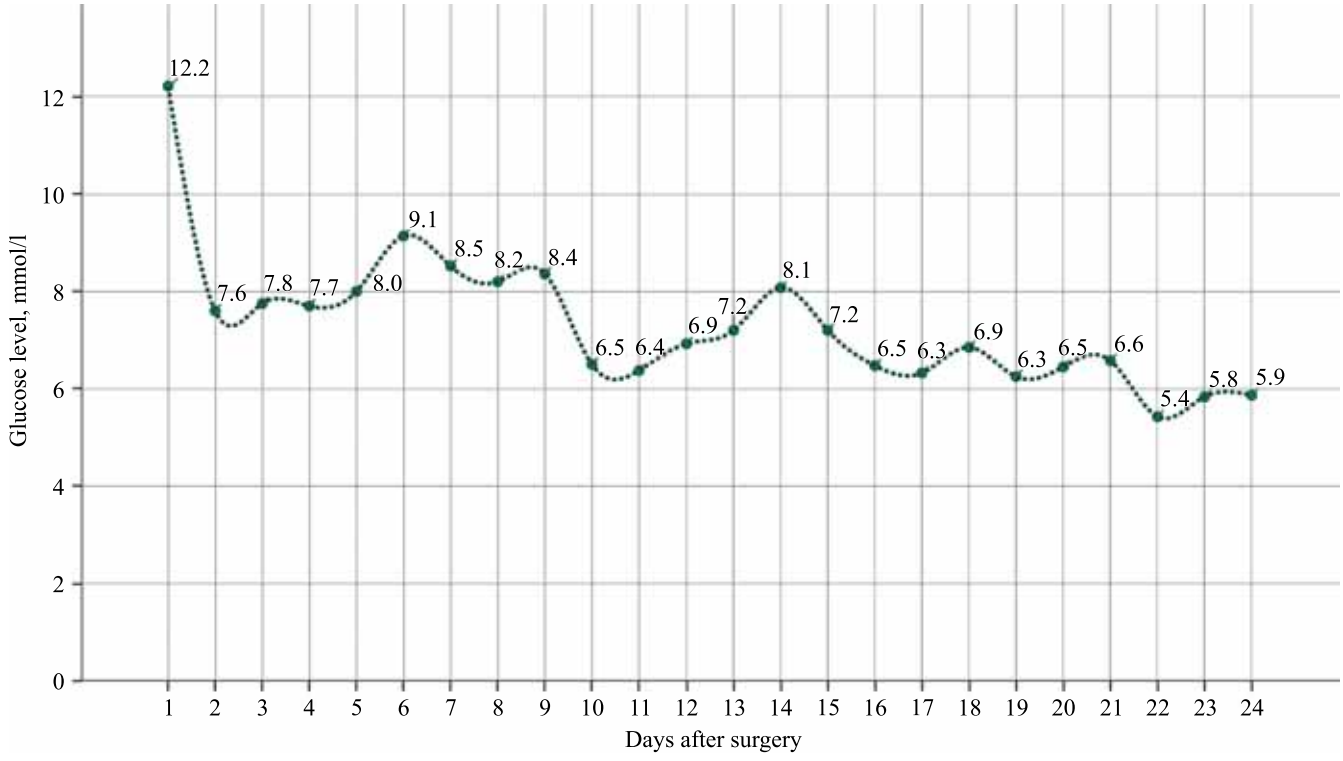

Fig. 7. Glycemia in the postoperative period

feeding the graft is visualized, its diameter in the anastomosis area with external iliac artery is up to 12 mm, the diameter of the artery is 5.5 mm. In the course of surgical intervention, there was a slight accumulation (up to 2 mm in thickness) of fluid over a distance of about 30 mm in the right iliac region.

The liver was not enlarged, the craniocaudal dimension was 148 mm. The liver parenchyma irregularly accumulates the contrast agent due to areas of hyperperfusion. Intrahepatic bile ducts and choledochus were not enlarged. The pancreas was not enlarged, with the following size: head 21 mm, body 12 mm, tail 16 mm; the duct of Wirsung was not dilated. Spleen was not enlarged in size, the structure was homogeneous. Adrenal glands were Y-shaped, located typically, not enlarged in size, and had a homogeneous structure. The kidneys were reduced in size, and parenchyma was sharply thinned. Renal excretory function was not visualized at 5 minutes. The loops of the large and partially small bowel were bloated with contents and gas. There were no pathological accumulations of fluid in the free abdominal cavity.

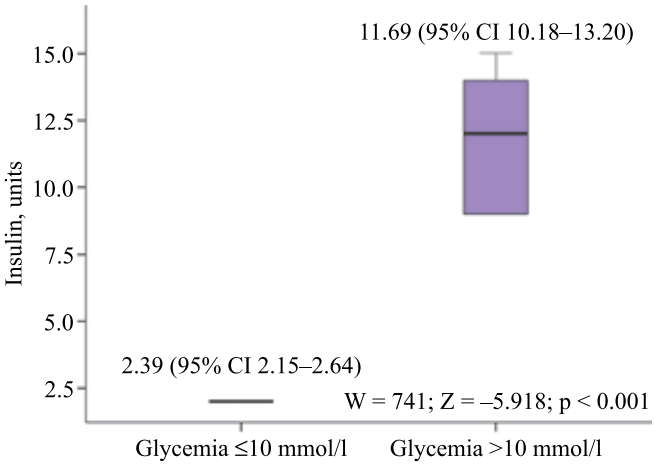

Fig. 8. Mean daily doses of insulin administered at minimum and maximum blood glucose elevations. 95% CI, confidence interval for the mean; p, significance of differences between indicators

As already noted, the patient had hypoglycemic episodes during the early postoperative period. The average glycemic level for the entire period of inpatient treatment was 7.52 mmol/l (95% CI 7.14–7.90). Elevated blood glucose levels were registered from day 5 to day 9 and from day 13 to day 15 (Fig. 7), which, of course, required adjusting the therapy and increased insulin doses, which we administered in such cases via an infusion machine (Fig. 8). The mean insulin dose administered during the entire postoperative period was 4.76 IU (95% CI 3.55–5.98).

On August 10, 2018, the patient was discharged from the hospital for outpatient treatment in a satisfactory condition, with recommendations on the timing of nephrologist monitoring and immunosuppressive therapy control.

At 4 years and 1 month after the operation, the patient's condition was satisfactory, she was socially

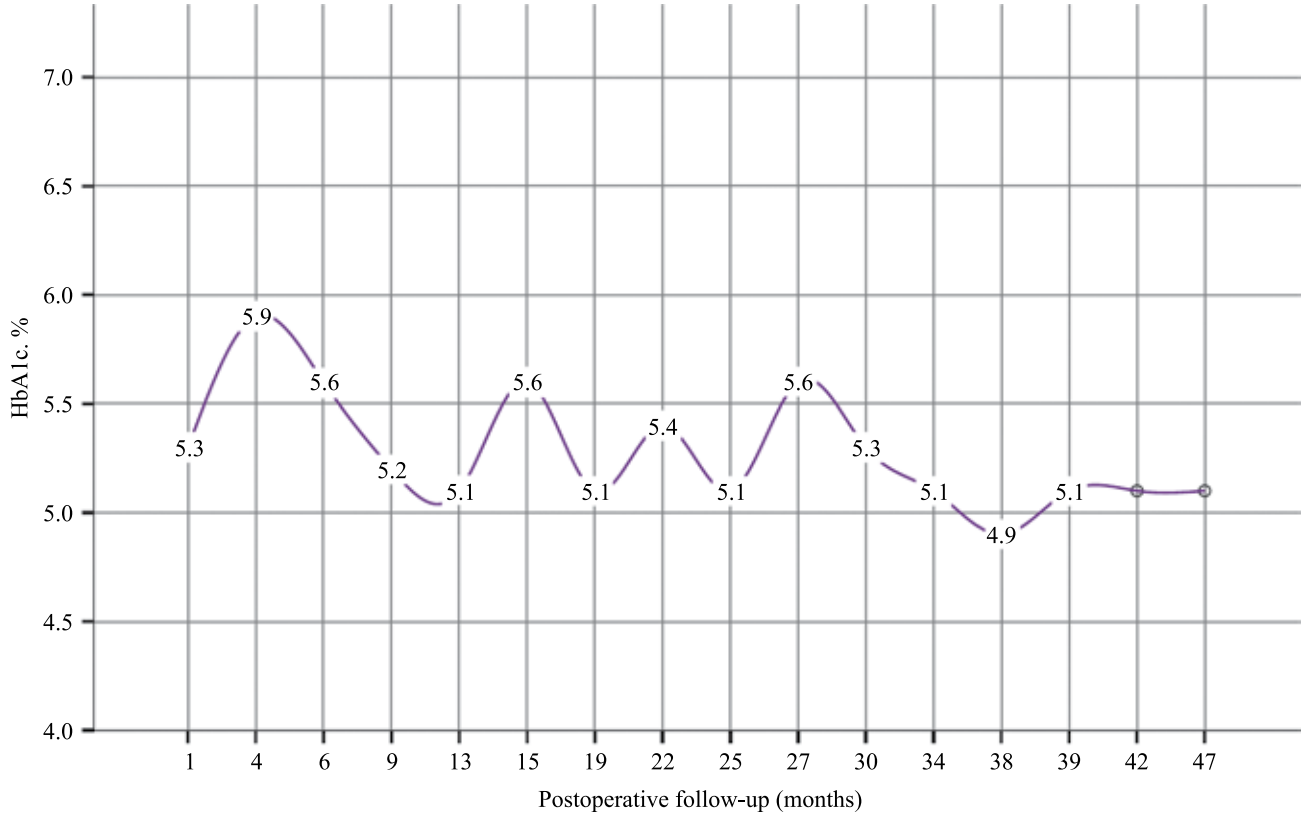

Fig. 9. Glycosylated hemoglobin in the long term after surgery

adapted, and there were no signs of graft dysfunction. The level of compensation for diabetes mellitus was regularly monitored (target laboratory values were achieved) (Fig. 9).

CONCLUSION

The presented clinical case clearly showed that the PTx technique developed by us can solve a number of urgent problems – graft preservation in case of purulent complications, and nonproliferation of purulent processes in the abdominal cavity. In spite of the fact that for 14 days, we noted inflammatory purulent discharge from the pancreas graft bed through the drains, which we regarded as acute graft pancreatitis, we were able to stop this complication with the help of conservative measures.

It should be noted that dynamic abdominal ultrasound examination during the whole post-transplant period showed complete intactness of abdominal organs in relation to inflammatory and purulent processes observed in the pancreas graft localization area. With a high probability, this condition could lead to more severe consequences. However, measures taken in advance (in terms of surgical tactics and transplantation technique) allowed to prevent graft loss and prevent the spread of purulent inflammation into the abdominal cavity and retroperitoneal space.

The surgical technique used allowed us to stop purulent inflammation in the transplant location area without repeated surgical intervention. This is especially important for this category of patients. The technique also allowed to obtain a long-term transplant effect.

Two pancreas and kidney transplants have been successfully performed in the surgical center using the original technique. The postoperative period was uneventful, the patients were discharged on days 13 and 17.

The authors declare no conflict of interest.

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CLINICAL CASE OF STAGED TREATMENT OF COMBINED COMPLICATIONS OF ORTHOTOPIC LIVER TRANSPLANTATION

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Hepatic artery thrombosis (HAT) following liver transplantation (LT) is a severe life-threatening complication that can lead to graft loss and mortality after LT. According to different reports, HAT incidence ranges from 2% to 9%. Modern endovascular and radiosurgical techniques allow for minimally invasive liver graft revascularization. Nonetheless, a major consequence of even a successful revascularization is ischemic cholangiopathy, which can lead to ischemic biliary strictures and anastomotic leak. The paper presents a clinical case of long-term complex treatment of combined complications of LT using minimally invasive endovascular and endoscopic techniques.

Keywords: orthotopic liver transplantation, biliary strictures, hepatic artery thrombosis, endobiliary stenting.

INTRODUCTION

Early HAT following LT is a devastating complication that can lead to graft loss and recipient death. According to different authors, the overall incidence ranges from 2% to 9% [1]. Arterial anastomosis is extremely important, but non-surgical risk factors for thrombosis, such as coagulation disorders against the background of decreased synthetic liver function, multiple arterial anastomoses and reconstructions, embolizations prior to transplantation, etc. are equally important [2–4].

Endovascular recanalization of the hepatic artery is considered the safest method of treatment. Modern radiosurgery techniques allow minimally invasive revascularization of the hepatic graft, including in cases of multiple attempts to form arterial anastomosis with recurrent thrombosis [5, 6].

A major consequence even after successful liver transplant revascularization is ischemic cholangiopathy, which can lead to ischemic biliary strictures and anastomotic leak [7]. Modern therapeutic tactics for biliary strictures correction provides for the priority of endoscopic methods of treatment and includes endoscopic retrograde cholangiopancreatography (ERCP) with subsequent installation of plastic stents [8–10].

In cases of ischemic cholangiopathy, it is not uncommon for long, complex strictures that are difficult to treat endoscopically to form. Prolonged external biliary tract drainage carries a risk of severe infectious complications and can be used in modern conditions for decompression in cases where it is not possible to recanalize stricture endoscopically [11]. The way out of such situations is the use of hybrid techniques including staged and simultaneous use of endoscopic and percutaneous drainage.

This paper presents a clinical case illustrating a multidisciplinary hybrid approach to the treatment of LT complications.

CLINICAL OBSERVATION

Patient J., female, 58. Liver cirrhosis on the background of chronic unverified hepatitis was first diagnosed in 2015. The patient received conservative therapy under the supervision of a hepatologist, with moderate positive dynamics. The disease worsened from February 2019 (Child–Pugh class C, MELD score 18–24). The patient was examined and put on the LT waiting list. Antithrombin III level at outpatient was 87%.

On October 25, 2019, orthotopic LT was performed using the piggy-back technique. Donor: male, 26 years old, diagnosed with brain death resulting from penetrating head injury. Primary cold ischemia time was 385 minutes, warm ischemia 40 minutes, and liverless period 55 minutes. End-to-end arterial anastomosis between the native hepatic arteries of the graft and the recipient was performed.

On October 26, 2019, follow-up ultrasound examination and subsequent CT angiography diagnosed HAT, and severe antithrombin III deficiency (38%) was detected in the laboratory.

On October 26, 2019, the patient underwent emergency angiography, recanalization and stenting of the hepatic artery (Aneugraft 4.0 × 27 mm stent graft). Two doses (1000 ME) of antithrombin III were administered.

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The postoperative period subsequently had no complications. The patient was transferred from the intensive care unit on day 4, she was discharged from the hospital on day 23 after the operation. Graft function was satisfactory. During outpatient follow-up, she received triple immunosuppressive therapy (tacrolimus, myfortic, prednisone), anticoagulants, and antiplatelets. After 6 months, follow-up angiography was performed – hepatic artery stent was passable (Fig. 1).

In April 2021, the patient noted gradual appearance and increase of bile-tinged skin, recurrent pain in the right subcostal area and increase in body temperature up to 38.6 °C. On April 27, 2021, the patient was admitted to the outpatient clinic. On admission to the hospital, marked biliary hypertension signs were revealed. According to ultrasound findings: right and left lobe ducts up to 14 mm, segmental ducts up to 5–8 mm, choledochus 16–17 mm. Blood flow in the liver graft was satisfactory. Also noteworthy was the rapid increase in laboratory signs of obstructive jaundice (Table). Magnetic resonance cholangiopancreatography (MRCP) showed multiple strictures in the biliary anastomosis area. There was encapsulated liquid mass (hematoma) in the subhepatic space (3.9 × 3.2 × 2.8 cm). There were no signs of liver graft structure and perfusion disorders (Fig. 2).

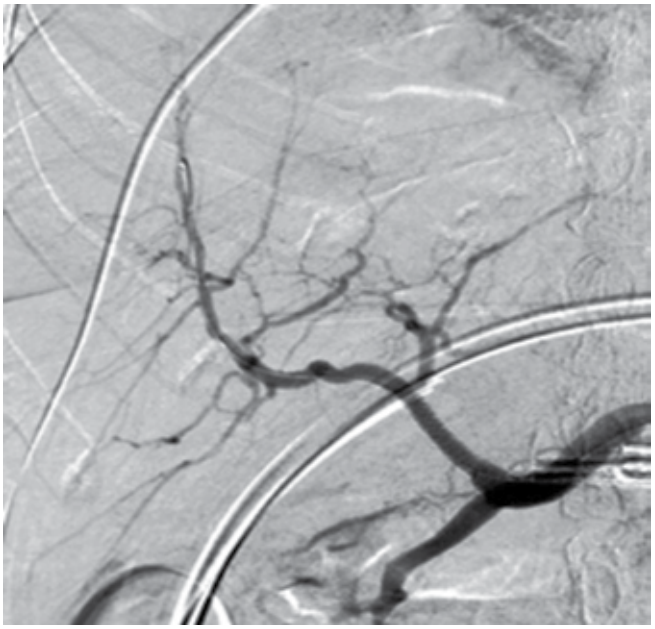

Fig. 1. Control angiography result 6 months after hepatic artery graft stenting

On April 30, 2021, an attempt at endoscopic recanalization was made. However, the guidewire could not be passed proximal to the stricture zone. Simultaneously, celiacography was performed, revealing up to 80% stenosis in the area of the previously installed stent (Fig. 2). Given the need for urgent resolution of biliary hypertension, the patient underwent ultrasound-guided percutaneous, transhepatic external drainage. Convincing positive ultrasound results (May 18, 2021 – up to 4 mm intrahepatic ducts, 10 mm choledochus) and laboratory dynamics were obtained after drainage (Table). Drainage of 400–700 ml bile daily. At the follow-up antegrade cholangiography, there was no contrast inflow that was distal to the biliary stricture zone (Fig. 2).

Given the available clinical data, after the patient's condition was fully stabilized, percutaneous transhepatic and endoscopic stenting of the biliary tract was performed on May 20, 2021 using the rendezvous method. Under hybrid operating room conditions, the constriction zone was recanalized antegradely with the help of an introducer and a guidewire; the guidewire was passed into the duodenum, grasped with endoscope forceps, replaced by a standard guidewire (4 m), and the common bile duct was stented with a 10 cm long, 8 Fr diameter plastic stent (Fig. 3).

The next step was balloon angioplasty of the restenosis area with a 4.0 × 20 mm catheter and hepatic artery stenting with a 4.0 × 32 mm drug-eluting stent (Promus) performed on May 26, 2021. Stent post-dilation at the level of its proximal part was done with a 4.5 × 15 mm high pressure balloon (Fig. 4).

Table

Dynamics of laboratory indicators

Indicator	27/04/2021	29/04/2021	01/05/2021	13/05/2021	24/05/2022	Units
Leukocytes	7.44	7.8	7.67	6.67	7.8	$\times 10^{9/L}$
Hemoglobin	131	123	94	92	93	g/L
Platelets	201	178	204	212	283	$\times 10^{9/L}$
Bilirubin	62	149	53.4	15	5.3	μmol/L
Direct bilirubin	–	141	38.8	–	–	μmol/L
ALT	493	742	462	52.7	20	U/L
AST	298	669	219	29.8	18.2	U/L
Creatinine	137	122	115	125	99	μmol/L
Urea	7.9	8.1	5.6	7.4	6.3	Mmol/L
INR	1.1	1.09	1.1	1.07	1.03	
Tacrolimus	4.7	–	6.7	7.4	6.1	ng/mL

The postoperative period was marked by a clear positive trend. Percutaneous transhepatic drainage was closed on day 6 and removed on day 14 after surgery. Follow-up MRCP revealed no filling defects, regression of biliary hypertension signs. The patient was discharged for outpatient treatment on day 10 after surgery.

Graft function was stable at month 12 after the surgical treatment. Laboratory results are presented in Table. The biliary stent was changed at months 5 and 10, and a nitinol stent is planned to be installed. Follow-up angiography found no signs of impaired hepatic blood flow at month 12 after restenosis correction.

DISCUSSION

Early HAT is a devastating complication that can lead to graft loss and patient death. High MELD scores, recipient age, prolonged warm ischemia time and hemostasis disorders are the most significant independent risk factors for HAT [5]. Minimally invasive endovascular restoration of arterial blood flow in the graft is the method of choice for surgical treatment in modern conditions and allows achieving a good outcome in most cases [12]. The use of drug-eluting stents carries a high risk of restenosis in the long term and, in our opinion, requires angiographic follow-up every 6 months. Based on world experience in coronary interventions, drug-eluting stents reduce the risk of restenosis significantly [13].

Ischemic cholangiopathy develops in more than half of patients with HAT [7]. Technical methods of endoscopic treatment of biliary strictures are diverse but extended; complex strictures of ischemic origin are often resistant to therapeutic endoscopy techniques [14, 15]. In such cases, hybrid techniques can be used. The use of plastic stents as the first-line solution for acute surgical problems seems to us justified. At the same time, the need to revise and replace plastic stents every 3–6 months has additional economic, technical and organizational challenges. Currently, there are ongoing discussions about the possibility of using self-expandable metallic stents (SEMS) in this situation. The main advantage of SEMS is that the number of required endoscopic interventions is reduced significantly [10]. The next stage in the described clinical case is the insertion of SEMS.

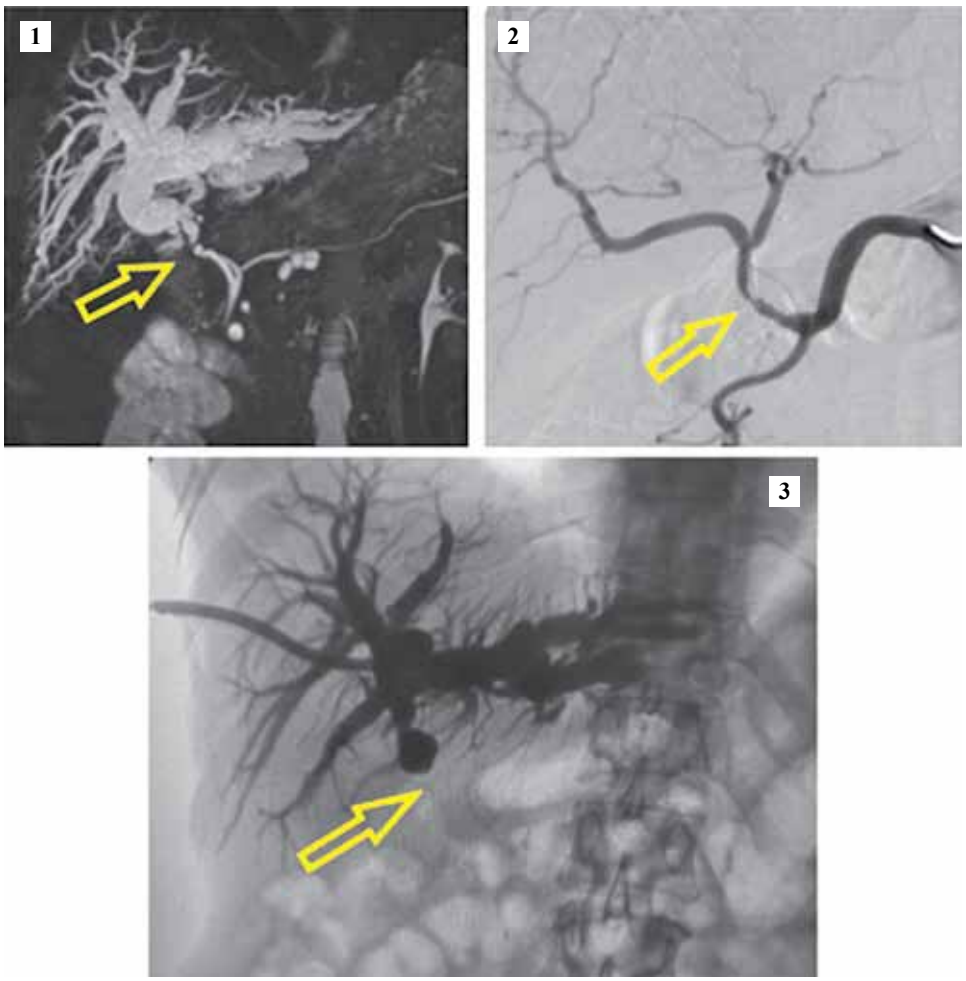

Fig. 2. Examination, percutaneous, transhepatic external drainage: 1, Magnetic resonance cholangiography (yellow arrow shows the stricture zone); 2, Celiacography (yellow arrow indicates the restenosis zone in the area of the previously installed stent); 3, Control antegrade cholangiography through external biliary drainage

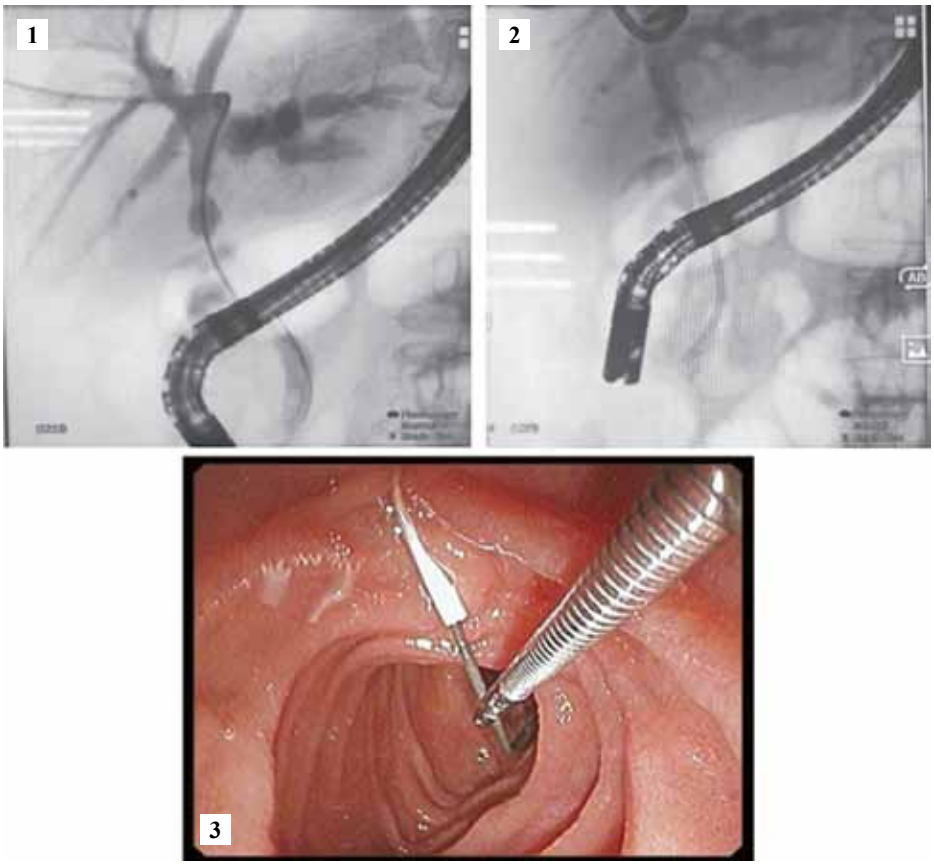

Fig. 3. Percutaneous transhepatic and endoscopic stenting of the biliary tract using the rendezvous technique: 1, recanalization of the constriction area and installation of a conductor; 2, installed plastic biliary stent; 3, endoscopic gripping of the conductor

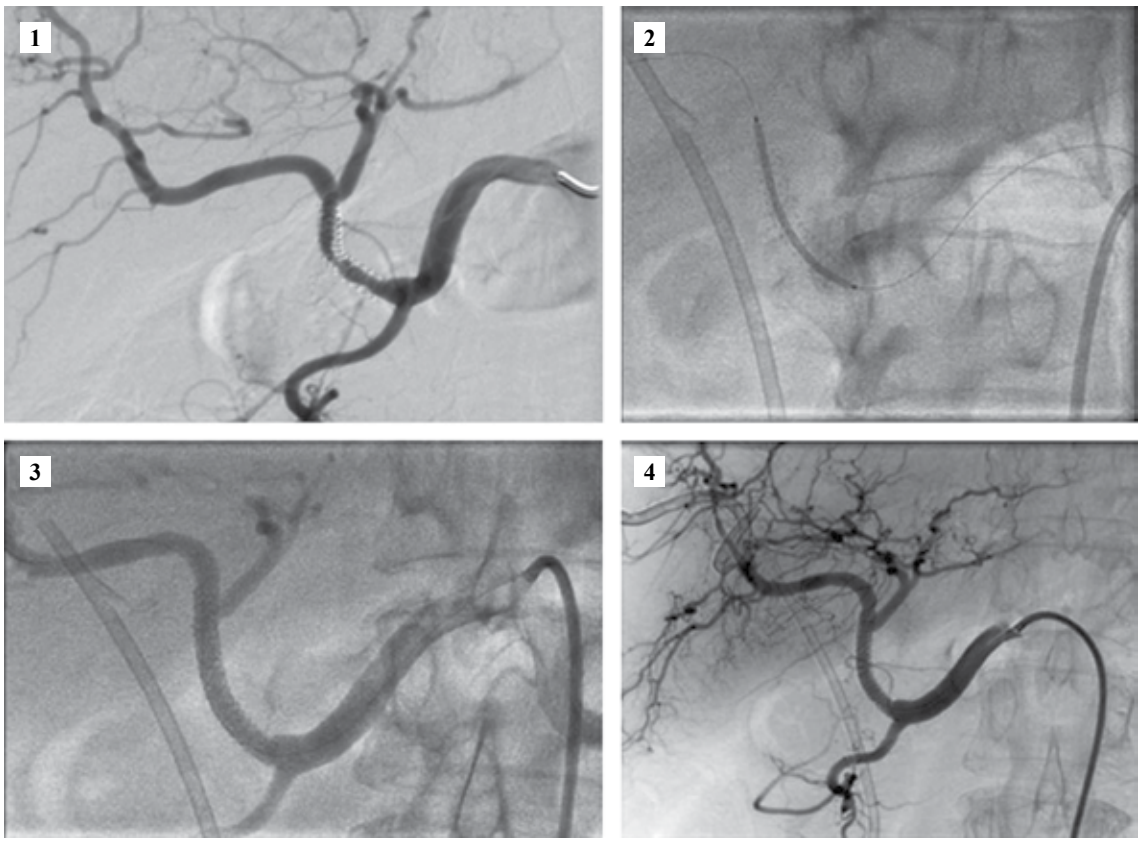

Fig. 4. Celiacography, balloon angioplasty and stenting of restenosis area: 1, restenosis of previously installed stent up to 80%; 2, balloon was inserted into the restenosis zone; 3, 4, stenting of the restenosis area with a 4.0 × 32 mm drug-eluting stent

CONCLUSION

Treatment of LT complications is a complex task that requires an integrated multidisciplinary approach and availability of a number of high-tech techniques at the transplantation center. Endobiliary and endovascular interventions provide ample opportunities in eliminating the consequences of impaired arterial blood flow in the liver transplant; in the long term, they can increase graft longevity. This clinical observation demonstrates the advantages and feasibility of staged minimally invasive correction of early and late combined complications of orthotopic LT.

The authors declare no conflict of interest.

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LIVING-RELATED KIDNEY TRANSPLANTATION: FIRST EXPERIENCE AT ST. LUKE'S CLINICAL HOSPITAL

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Kidney transplantation (KT) is regarded as the most effective therapeutic approach for people with end-stage renal disease. However, for a number of reasons – constant increase in the incidence of diseases contributing to formation and development of chronic kidney disease, as well as continuing shortage of donor organs – 78–95% of patients in need of a kidney transplant do not receive the necessary treatment, and the waiting list stretches for several years. This paper presents the first outcomes of KT for chronic glomerulonephritis performed at St. Luke's Clinical Hospital in St. Petersburg, in collaboration with the staff of Shumakov National Medical Research Center of Transplantology and Artificial Organs.

Keywords: living-related kidney transplant, chronic glomerulonephritis.

INTRODUCTION

Today, kidney transplantation (KT) is the most effective renal replacement therapy (RRT) modality for end-stage chronic kidney disease (CKD) [1, 2]. Unlike other RRT methods (hemodialysis and peritoneal dialysis, hemodiafiltration), providing only removal of low-molecular-mass toxins and metabolites, as well as excess water, the transplanted kidney performs all the functions of the affected organ – urinary, detoxification, metabolic, biosynthesis of hormones and neurotransmitters, other bioactive substances, etc.

Over the past 2021, 1384 kidney transplants were performed in our country; the number of deceased and living (related) donors was 1183 and 201, respectively. At the same time, 6313 potential recipients were included in the 2021 waiting list. Consequently, in 2021, only 21.9% of patients who needed transplantation from the 2021 waiting list received a kidney transplant [3, 4].

According to the Registry of the Russian Transplant Society, the absolute number of annual KTs increased 2.5-fold from 2006 to 2021 [3, 4]. During the same observation period and to approximately the same degree, the absolute number of persons on the waiting list also increased, while the average waiting time for KT practically did not change. As a result, over the past 15 years, the annual need for KT has remained unmet in approximately 78–95% of patients with end-stage CKD [4]. The reasons for the discrepancy between the demand and possibilities of transplantation care include, firstly, a constant increase in the incidence of type 2 diabetes mellitus, bronchial asthma, hypertension, coronary heart disease, chronic heart failure and other conditions contributing to the formation and development of CKD; secondly, persistent shortage of donor kidneys [1, 3, 4].

In 2019, our country approved and adopted the departmental target program "Organ donation and transplantation in the Russian Federation"; it was aimed at increasing the availability of medical care through human organ transplantation. Implementation of the program began on December 29, 2021, at St. Luke's Clinical Hospital in St. Petersburg. In May 2022, the first kidney allotransplantation from a related donor was successfully performed. The article presents the first results of the approved program.

The paper presents the first outcomes of related kidney transplantation in chronic glomerulonephritis performed at St. Luke's Clinical Hospital, St. Petersburg, jointly with the staff of Shumakov National Medical Research Center of Transplantology and Artificial Organs.

CASE DESCRIPTION

A 34-year-old man complained of recurrent high blood pressure (BP) up to 140–150/80–90 mmHg (against the background of continuous use of nifedipine (10 mg in the morning) and bisoprolol (25 mg in the evening), he had usual blood pressure of 120–130/80 mmHg), tendency

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to a fall in body weight from 54 to 49 kg over 2–3 years. When collecting the medical history, the man completely denied the existence of nephrological diseases in his relatives. With regard to himself, he noted that in childhood he underwent cystoscopy prescribed for chronic pyelonephritis or cystitis, that he suffered from angina at the age of 27, which, according to the patient, was complicated by acute glomerulonephritis (he remembers that there was pain in his lumbar region, was treated by a therapist, was consulted by a nephrologist, the issue of kidney biopsy was discussed, which was not performed because the patient unilaterally stopped visiting medical specialists). Five years after that, skin itching suddenly appeared. On this occasion, he was examined by a therapist, a gastroenterologist, and a dermatologist. Atopic dermatitis was diagnosed, treatment was prescribed with positive effect. He remembers that the doctors talked about "elevated creatinine level" (the patient later found his old tests, creatinine levels from 2019 was 193 μmol/l). One and a half years later, he had episodes of symptomatic arterial hypertension and associated headaches; he was examined by a nephrologist, who diagnosed chronic glomerulonephritis and stage 4–5 CKD. The doctor prescribed medication therapy (nifedipine 10 mg in the morning, bisoprolol 2.5 mg in the evening, polysorb 1 tbsp. per day, milurit 100 mg (1 tablet, due to the identified hyperuricemia) every other day, aquadetrim 2 drops per day). Other medical history details: at the age of 8, he had surgery for unilateral cryptorchidism; at the age of 32, chronic hepatitis B with minimally pronounced biochemical and histological activity was detected accidentally during examination. Objectively: general state was satisfactory, skin was dark-colored, dry, mild hyperpigmentation in the nipple area, funnel-shaped chest deformity, pronounced thoracic kyphosis. Breathing in the lungs was vesicular, no wheezing was heard, respiratory rate was 15 per minute. Heart sounds were clear, rhythm was correct, heart rate (HR) and BP were 68 bpm and 140/95 mmHg, respectively. The tongue was moist and clean, the abdomen was soft, painless on palpation, and the liver edge was not palpable. Bowel and bladder functions were not violated.

Preoperative examination included assessment of the cardiovascular system (ECG, daily ECG and BP monitoring, echocardiography and Doppler echocardiography, duplex scanning: a) head and neck vessels, b) arteries and veins of the lower extremity, consultations with a cardiologist and a cardiovascular surgeon), respiratory system (lung radiography, spirography, spirography with a bronchodilator test), gastrointestinal tract (esophagogastroduodenoscopy), urinary system (multispiral CT scan of kidneys and urinary tract, consultation with nephrologist), endocrine glands (determination of serum levels of glucose, ionized and total calcium, phosphorus, parathyroid and thyroid hormones, examination by an endocrinologist), the nervous system (examination by a neurologist and a psychiatrist), ENT organs and organs of vision, as well as a comprehensive ultrasound of the liver, gallbladder, pancreas, spleen, kidneys, and laboratory blood and urine tests (cellular and biochemical compositions, blood coagulation and anticoagulant activity, determination of HBsAg antigen (HBsAg) to hepatitis B virus in blood, bacteriological analysis of urine for aerobic and facultative anaerobic microorganisms), identification of recipient antibodies to the donor's major histocompatibility complex.

Preoperative examination results indicated hypochromic anemia due to a decrease in circulating red blood cell count to 3.77 × 10^{12}/l and a hemoglobin levels of up to 106 g/l, microhematuria and proteinuria (0.5 g/l) in the urine, hyperazotemia due to increase in serum uric acid, urea and creatinine levels to 425.6 μmol/l, 36.2 mmol/l and 493.6 μmol/l, respectively, decrease in glomerular filtration rate (GFR) to 10.76 ml/min/1.73 m^2, increase in serum α-amylase activity to 128.0 U/l, ultrasound signs of parenchymal cysts and nephrosclerosis of the right and left kidneys, CT scan signs of diffuse thinning of the parenchyma of both kidneys, in addition: 1) electrocardiographic – sinus bradycardia and local intraventricular conduction disorders; 2) adhesive left-sided otitis media without exacerbation; 3) hyperglycemia up to 5.85 mmol/l; 4) mild myopia, hypertensive retinal angiosclerosis.

Based on the data obtained during diagnostic activities, the council of specialists made the following diagnosis: main diagnosis – chronic glomerulonephritis; complications of the main diagnosis: stage 5 CKD (GFR 10.76 ml/min/1.73 m^2), hyperphosphatemia, hyperuricemia, chronic nephritic syndrome without exacerbation, stage 1 secondary hypertension, mild anemia; concomitant diagnosis: mild myopia, hypertensive retinal angiosclerosis, chronic hepatitis B infection without activity, pre-dialysis patient. The council of specialists decided that RRT was necessary.

When choosing the RRT technique, we took into account the desire of the patient's blood relative (mother) to become a kidney donor for the patient. Also considered were the results of examination of this potential related donor, which, firstly, showed no contraindications for donor nephrectomy; secondly, multispiral CT scans showed signs of right renal artery tripling with a branching to the upper segment parenchyma of the proximal accessory vessel into the renal hilum – main and distal accessory vessels with a late proximal division.

Single-port left laparoscopic nephrectomy (LESS, laparoendoscopic single-site surgery) in the donor was performed in the right-side position under endotracheal general anesthesia (Fig. 1).

Laparoscopic port access was performed 2 cm above the umbilicus. After CO_2 insufflation, a single-port (Nelis, Kyung-gi, Korea) was installed. Next, the descending colon was mobilized, then using the ThunderBeat

Fig. 1. Single-port left laparoscopic nephrectomy in the donor

instrument (Olympus, Japan), the kidney was isolated from fatty tissue from all sides. The renal artery and vein below the level of the aorta were treated selectively. Two Hem-o-Lok clips (Teleflex, Mexico) were placed on the renal artery, then the vessel was cut off using scissors. As low as possible to the junction of the inferior vena cava and renal vein, the renal vein was similarly crossed. The ureter was isolated to the lower third, crossed with a Hem-o-Lok clip. After desufflation and incision (8 cm) in the continuation of trocar access, the abdominal cavity was opened in layers, the kidney and part of the upper third of the ureter were removed in one block. The intervention was completed with hemostasis control (dry), layer-by-layer suturing and iodine treatment of the wound, application of aseptic dressing. The kidney was placed in cold preservation with Custodiol solution (solution volume was 1 liter, Dr. Franz Kjoller Chemie GmbH, Germany); static cold preservation lasted for 35 minutes (Fig. 2).

Allotransplantation of donor (related) kidney to the recipient on the right (left kidney) and stenting of the left ureter was performed under endotracheal anesthesia with muscle relaxants; the operation started with bladder drainage with a Foley urethral catheter No. 16, then bladder was filled with 0.9% sodium chloride solution in 100 ml volume. After treating the surgical field with an oblique typical incision in the right iliac region, the retroperitoneal space was accessed in layers, the lower epigastric vessels were isolated, ligated and crossed, and the spermatic cord was taken aside. Then the external iliac artery (EIA) and external iliac vein (EIV) were isolated and mobilized. The kidney graft, which had one artery up to 4 cm in length, one vein and one ureter, was placed in the wound, clamps were placed on the EIV and venotomy of up to 2.5 cm was performed. Vascular anastomoses were made between: 1) the graft vein and the EIV by end-to-side continuous sutures with Prolene 5/0 suture (Johnson & Johnson, USA); 2) between the graft artery and the EIA by end-to-side continuous sutures with Prolene 6/0 suture. At the start of blood flow (180 minutes from the beginning of intervention), the kidney turned pink, acquired satisfactory turgor; inflow of urine in infrequent drops was noted, Lich-Gregoir ureteroneocystostomy anastomosis using PDS 5/0 suture (Johnson & Johnson, USA), with ureteral stenting with 12 cm, 7 Fr internal ureteral stent was placed. The operation was completed by controlling hemostasis (dryly), washing the wound with a 1% solution of povidone-iodine, establishing a closed drainage into the retroperitoneal space through a counter-opening in the upper corner of the wound, layer-by-layer suturing of the wound, and applying an aseptic dressing. Separately, we note the following: the graft was transplanted into the right iliac region retroperitoneally. From the moment the incision was made in the right iliac region, basiliximab (20 mg) and prednisolone (500 mg) were injected intravenously in order to prevent acute graft rejection, followed by the use of a standard triple-combination immunosuppressive therapy regimen, including tacrolimus, mycophenolate mofetil, and methylprednisolone. Transplant functioning was accompanied by normalization of nitrogenous metabolism on postoperative day 2 (decrease of serum urea levels from 36 mmol/l to 5.6 mmol/l and creatinine from 493.6 μmol/l to 104.5 μmol/l); and by change of

Fig. 2. Graft preparation: explanted graft is placed in ice chips after custodiol perfusion

daily diuresis from 6000 ml by urethral catheter within 48 hours after the intervention to 1540 ml on postoperative day 3. Postoperative immunosuppressive therapy was provided on days 0 and 4 by administration of basiliximab (20 mg), tacrolimus (2 mg, 2 times a day with subsequent correction depending on tacrolimus serum levels), mycophenolate mofetil (1000 mg, 2 times a day), methylprednisolone (16 mg, in the morning). The patient was discharged on day 12 after surgery in a satisfactory condition.

There were no surgical complications in the donor after the intervention. The right kidney function was fully restored on day 1 of the postoperative period.

CONCLUSION

The presented clinical case demonstrates a successful solution to problems associated with expansion of high-tech care for St. Petersburg residents.

The introduction of a new type of high-tech care for patients at St. Luke's Clinical Hospital will increase the volume of transplant care for the residents of St. Petersburg and Leningrad Oblast in general.

The authors declare no conflict of interest.

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LAPAROSCOPIC PARTIAL NEPHRECTOMY IN ALLOGRAFT KIDNEY FOLLOWED BY INTRARENAL URINARY TRACT RECONSTRUCTION AND URETERAL REIMPLANTATION (CLINICAL REPORT)

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This paper presents a clinical case of laparoscopic nephrectomy for a large (10 cm) renal sinus mass in an allograft kidney, followed by intrarenal urinary tract reconstruction with ureteral reimplantation. The surgery had an acceptable oncological outcome, without loss of kidney function. Regardless of the volume and extent of the tumor process, the use of minimally invasive, nephron-sparing treatment techniques takes a leading position in the treatment of renal cancer in kidney recipients. Intrarenal urinary tract reconstruction allows a kidney to be saved even if the tumor is significantly large and/or inoperable.

Keywords: kidney graft masses, transplant, partial nephrectomy.

INTRODUCTION

To date, no objective statistical data on the detection rate of kidney graft cancer (KGC) are reflected in the national and world literature, due to the rare disease incidence. However, a detailed review of one of the largest meta-analyses by Griffith J.J. et al. devoted to solid renal masses in transplant allograft kidneys reflects the incidence of renal cell carcinoma (RCC) in transplant patients, which is from 0.19 to 0.5%. Compared with the non-transplant population (0.017%), this figure is already a 10-fold increase [1–3].

At the moment, the range of methods used for the treatment of renal masses in a kidney graft is similar to the approaches in the treatment of renal parenchymal cancer. The frequency of local recurrence after surgical treatment of a kidney graft is the same as in the population of patients without transplantation. The morphological profile of renal parenchymal cancer in a kidney graft is quite diverse and includes clear cell RCC (45.7%), papillary RCC (42.1%), chromophobe RCC (3%), and other forms of renal cancer (9.1%) [1–3].

Tumors were managed by partial nephrectomy (67.5%), radical nephrectomy (19.4%), percutaneous radiofrequency ablation (10.4%), and percutaneous cryoablation (2.4%). Due to the rarity of this disease, mainly open-access kidney graft resections have been described in the literature; only in 2020 was transperitoneal laparoscopic partial nephrectomy described [4] for a 28×24-mm renal mass in an allograft kidney. Also, literature features single cases of robot-assisted transperitoneal laparoscopic partial nephrectomy for masses in an allograft are described. However, there is no clear standard in the technique of surgical assistance and the type of graft ischemia [5, 6].

Thus, there are currently no clear clinical guidelines for the treatment of renal masses in transplanted allograft kidneys. The clinical cases described are scattered.

It is worth noting the functional and oncological outcomes of organ-preserving operations performed. Of the 80.3% of patients who underwent nephron-sparing interventions, 7.6% returned to dialysis, 6.1% developed tumor recurrence within 2.85 years, 3.6% developed local recurrence over a mean follow-up of 3.12 years [1–3].

This paper presents a clinical case of a renal sinus tumor in an allograft kidney for which laparoscopic partial nephrectomy and subsequent intrarenal urinary tract reconstruction were performed.

An ultrasound examination of the patient (female, 33 years old) revealed a renal tumor of the allograft kidney, which was confirmed by MRI (Fig. 1); the size of the mass was 10.3 × 8.3 cm, without distinct instrumental evidence of distant focal pathology.

The patient's medical history shows that organ transplantation was performed 4 years before the tumor was detected. After transplantation, the patient received immunosuppressive therapy with calcineurin inhibitors, mycophenolate and corticosteroids.

MATERIALS AND METHODS

Laparoscopic nephrectomy with intrarenal urinary tract reconstruction and ureteral reimplantation was performed.

Kidney graft mobilization was the first stage. The second stage was isolation of the right external iliac artery (EIA) (Fig. 2, a), taken on a holder; after mobilization of the mass, the mass resection boundaries were deter-

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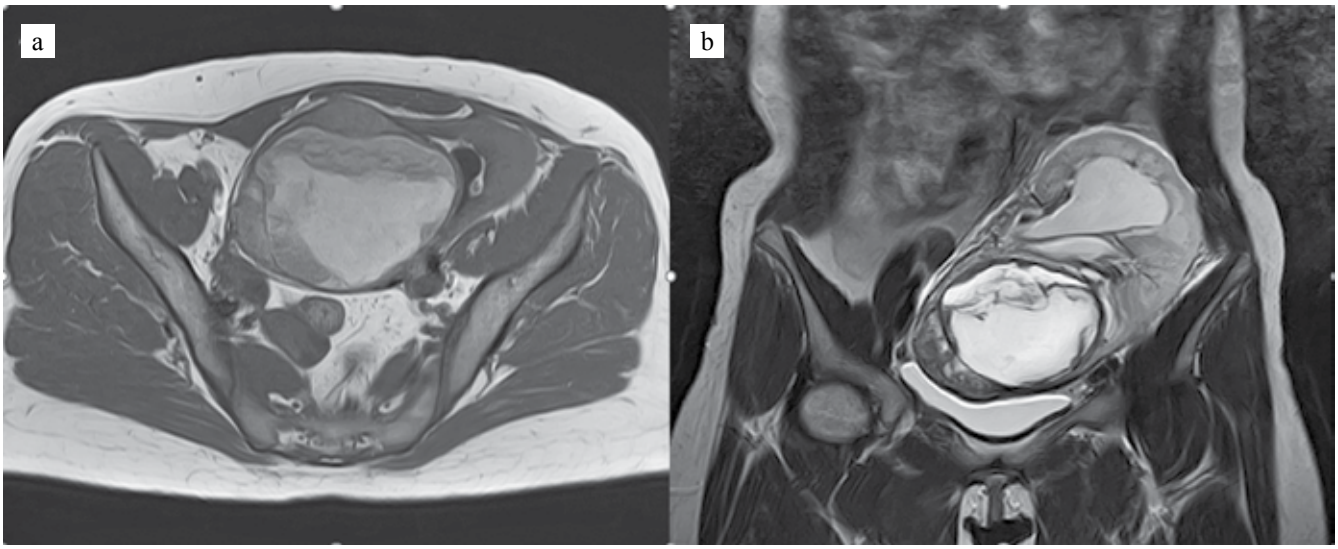

Fig. 1. MRI of an allograft kidney with renal sinus tumor: a, axial image; b, frontal image

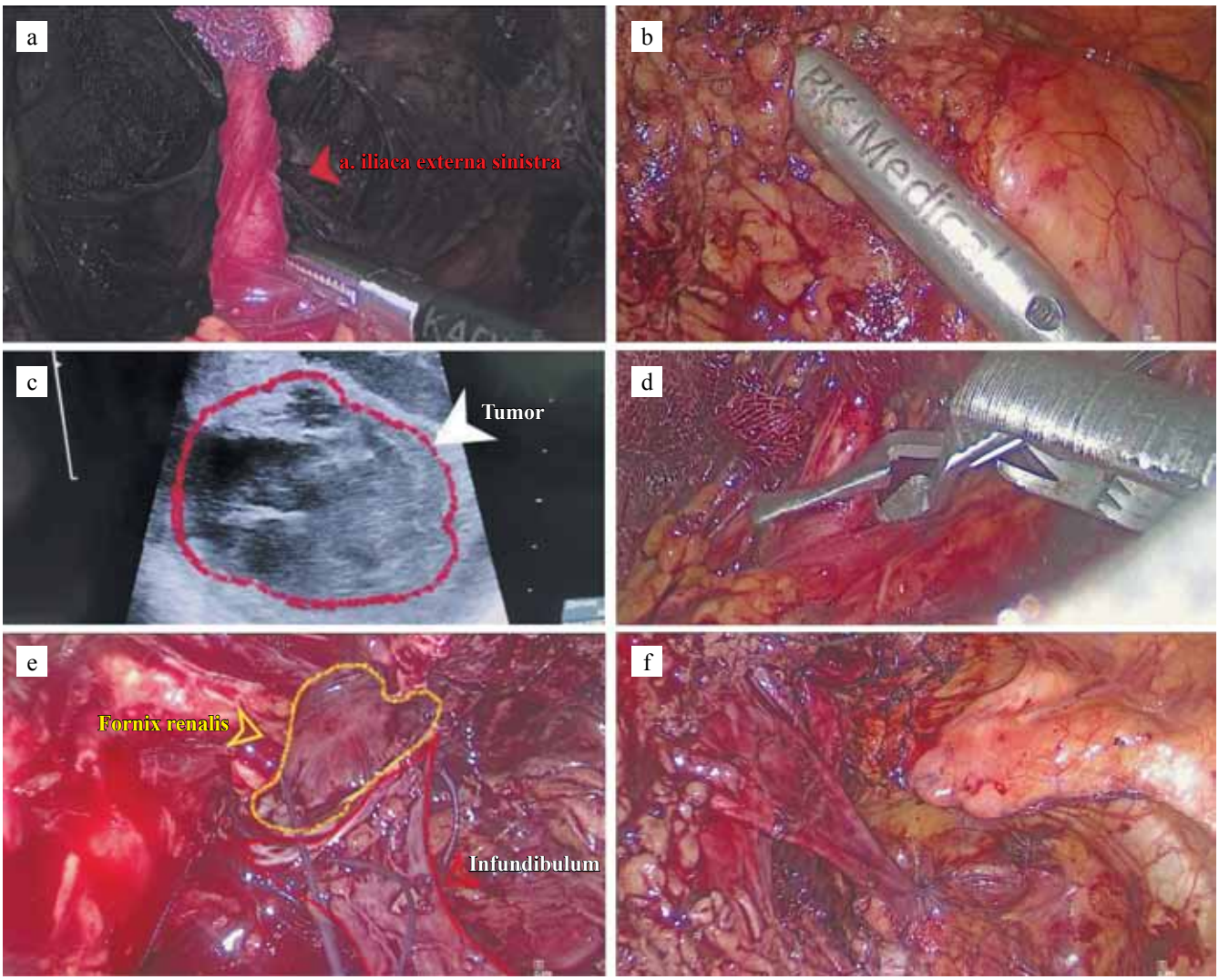

Fig. 2. Stages of laparoscopic partial nephrectomy for allograft tumor and urinary tract reconstruction. a, dedicated right external iliac artery (*a. iliaca externa sinistra*); b, performing intraoperative ultrasound examination; c, defining the tumor borders; d, clamping the external iliac artery; e, suturing the small calyx to the renal papilla with separate nodal sutures; f, view of ureterocystoneoanastomosis

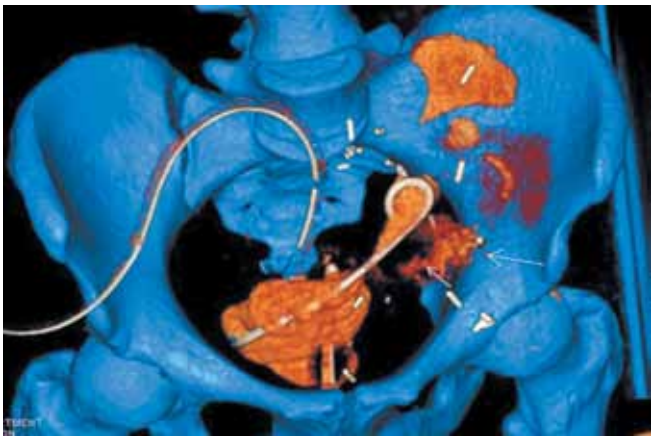

Fig. 3. CT excretory urography on day 2 after surgery

mined (Fig. 2, c) using an ultrasound endoscopic probe (Fig. 2, b); after clamping the EIA (Fig. 2, d), tumor enucleation was performed, while noting the linear calyx defects and complete calyx resection; the renal papilla was separately visualized, the ureter was cut off from the bladder. The small calyx was sutured to the renal papilla with separate nodal sutures (Fig. 2, e), and the linear calyx defects were sutured.

After hemostasis control, additional sutures were used to restore the anatomical shape of the renal graft. The clamp was removed from the EIA, there were no signs of bleeding. Ischemia time was 40 minutes. After that, ureterocystoneoanastomosis was performed (Fig. 2, f). The operation was completed by draining the pelvis and abdominal cavity with drains. The operation time was 380 min.

RESULTS

The postoperative period was uneventful. CT excretory urography on day 2 after the operation (Fig. 3); there was no evidence of urinary leakage.

The drains were removed on day 5 after surgery. Creatinine levels before surgery and on the day of discharge were 174 μmol/l and 130 μmol/l, respectively. The patient was discharged home after 10 days of hospitalization. During the 12-month follow-up, no distant focal pathology was detected during a comprehensive follow-up examination; no progression of renal failure was noted as well.

CONCLUSION

Although laparoscopic nephrectomy, particularly in transplanted kidneys, appears to be less popular than robot-assisted surgeries for kidney cancer, it is a cost-effective method that can be safely performed in experienced hands, even in extremely difficult cases. Intrarenal urinary tract reconstruction allows to save a kidney even if the tumor is significantly large and/or inoperable. In addition, it can also be performed in kidney transplant recipients.

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The authors declare no conflict of interest.

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KIDNEY TRANSPLANTATION IN A PATIENT WITH FAMILIAL MEDITERRANEAN FEVER COMPLICATED BY SECONDARY AMYLOIDOSIS (CLINICAL REPORT)

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The paper presents a clinical case of successful kidney transplantation (KTx) in a patient with end-stage chronic kidney disease (ESKD) resulting from familial Mediterranean fever (FMF). Pre-transplant preparation and post-transplant management tactics are presented. The authors conclude that ESKD can be effectively treated by KTx in a patient with FMF against the background of ongoing pathogenetic therapy in autoinflammation.

Keywords: familial Mediterranean fever, amyloidosis, chronic kidney disease, kidney transplantation.

Familial Mediterranean fever (FMF), also known as recurrent polyserositis, is a hereditary monogenic disease with an autosomal recessive transmission mechanism. It has an autoinflammatory nature and characterized by recurrent sudden fever attacks [1] combined with a significant increase in the level of acute phase markers (erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), serum amyloid A1 (SAA1) [1–3]. Four clinical variants of FMF depending on the localization of inflammation are distinguished: abdominal (95.3%), thoracic (28.0%), articular (37.3%) and febrile (16.7%) [4].

FMF is caused by point mutations within the Mediterranean fever (MEFV) gene. Currently, more than 29 MEFV mutations have been reported, of which three mutations account for over 90% of FMF cases – M680I (found in most cases in Armenians), M694V and V726A [4].

The clinical manifestations of FMF are due to the biological effects of pyrin, a protein encoded by the MEFV gene. Mutated pyrin induces increased inflammatory response mediated by interleukin-1 (IL-1). Along with episodes of typical or atypical FMF attacks, the disease can be subclinical in nature, which, like manifest forms, can lead to the most severe complication of FMF – AA amyloidosis – which determines the prognosis for life in people with this autoinflammatory syndrome [5]. The main factor in the development of AA amyloidosis (reactive or secondary) in the development of an inflammatory response is an up to 1000-fold increase in serum SAA levels [6–8].

Amyloidosis is the most formidable complication of FMF, in which fibrillar glycoprotein amyloid is deposited in tissues and organs [8]. The leading manifestation of amyloidosis in Amyloidosis is the most formidable complication of FMF is involvement of kidneys in the pathological process, with the lesion of which the proteinuric, nephrotic and uremic stages are distinguished [4]. In clinical guidelines on diagnosis and treatment of systemic amyloidosis, the main strategy of AA amyloidosis treatment is effective suppression of inflammation until normalization of the levels of acute phase inflammatory markers – CRP and/or SAA [8, 9].

Colchicine is the drug of choice for FMF treatment, while in about 15–20% of patients, colchicine is initially ineffective. In this case, the current understanding of FMF pathogenesis allows proposing alternative approaches based on anticytokine therapy, primarily by IL-1 inhibitors [4].

Cases of kidney transplantation (KTx) in patients with FMF and secondary AA amyloidosis have been described in foreign literature [9–12]. We have not found any published cases of KTx in patients with FMF in the Russian literature. Studies describe the effectiveness of KTx for systemic amyloidosis (five-year survival rate of kidney and graft recipients is more than 60%); amyloidosis occurs in a transplanted kidney in about 30% of patients; it is the cause of graft loss in only 2–3% of patients [8].

Objective: to present a clinical case of KTx in a patient diagnosed with FMF complicated by secondary AA amyloidosis.

CLINICAL CASE

The anamnesis of patient C., born in 1974 (Armenian, female) shows that the first signs of FMF were revealed at the age of two in the form of ankle joint pains. At the

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age of 4, after suffering from hepatitis A, her articular syndrome was followed by fever syndrome (attacks lasted for 2–3 days and resolved on their own); at 9, she had abdominal syndrome; at 10, thoracic syndrome occurred. Until the age of 18, the patient continued to be disturbed by episodes of fever for 3–4 days, which resolved on their own. Until the age of 18, due to the prevalence of articular syndrome, the patient was repeatedly treated in rheumatology units, where she was diagnosed with systemic lupus erythematosus with joint disease, as well as reactive polyarthritis, systemic vasculitis with joint disease, abdominal syndrome.

At the age of 20, the patient's regular examination revealed hepatosplenomegaly, urinalysis showed proteinuria up to 0.2 g/day. Clinical blood tests showed decreased hemoglobin levels and elevated ESR. FMF was diagnosed after studying the patient's history and clinical data. Colchicine, 2 mg/day, was prescribed as baseline therapy. Tolerability of therapy was satisfactory. Against the background of treatment, the frequency of seizures decreased to once a year. The severity of seizure symptoms also decreased.

In order to confirm the diagnosis morphologically, liver biopsy was performed in 1997 at the age of 23, where no amyloid was detected. A biopsy of the rectal mucosa revealed an amyloid deposit. In 2004, at the age of 30, the diagnosis of recurrent disease was genetically confirmed, MEFV mutations (M694V, V726A, M680I, F479L, E148Q, M694I, R761H) were detected, one of the mutations was found in a homozygous state.

From 2006 to 2016, there were no clinical manifestations, but there were still changes in the tests in the form of increased CRP, ESR, which indicated chronic subclinical inflammation.

In 2016, at the age of 42, another exacerbation of the disease occurred against the background of stress. Examination revealed, for the first time, shortness of breath during physical exertion, marked lower extremity edema, increased transaminases, decreased albumin level with normal total protein level and 10-fold increase in CRP. The therapy was corrected, colchicine dose was increased to the maximum tolerated dose – 6 mg/day. In the same year, due to progression of AA amyloidosis against the background of the recurrent fever, the patient developed nephrotic syndrome resistant to treatment with colchicine, the patient was recommended genetically engineered therapy with monoclonal antibodies to interleukin-1β (canakinumab, registered in the Russian Federation).

In July 2019 (45 years old), at the next follow-up of laboratory data, creatinine levels increased to 164 μmol/L, renal filtration function decreased to 43 ml/min/m^2 according to CKD-EPI. Progression of the pathological process was noted, manifested as increase in ESR to 70 mm/hour, increase in CRP to 47 mg/l, decrease in albumin level to 26.3 g/l, with normal total protein at 70.9 g/l, increase in daily proteinuria to 4.8 g/day. Anti-interleukin-1β monoclonal antibody (canakinumab) was administered subcutaneously 150 mg once every 4 weeks, low-molecular-weight heparins were added to the therapy. Laboratory parameters stabilized during the treatment: decrease in CRP to 1.1 mg/l, decrease in ESR to 15 mm/h, and decrease in daily proteinuria to 1.1 g/day. The patient was completely relieved of clinical symptoms.

In January 2020 (age 46), the patient had a mild SARS-CoV-2 infection with a minimal percentage of lung affection (5%). Eight months later, there were increased shortness of breath, increased lower extremity edema, and poor correction of arterial hypertension. Tests revealed an increase in creatinine levels to 570 μmol/L (CKD-EPI 7 mm/min/1.73 m^2), urea to 32 mmol/L, and potassium to 6.04 mmol/L. Total protein levels remained high (82.4 g/l) with 4 g/day daily proteinuria. The patient started renal replacement therapy by long-term hemodialysis.

In September 2021, in the absence of signs of chronic autoinflammatory process, the patient was put on the kidney transplant waiting list. She continued to receive pathogenetic therapy: colchicine 1 mg/day and canakinumab 150 mg once every 4 weeks. Against the background of the therapy, CRP level remained at 2 mg/l, ESR at 20 mm/hour.

Kidney transplantation and early postoperative period

At the age of 48 (March 19, 2022), the patient underwent allotransplantation of a kidney obtained from a deceased donor. Graft function was immediate, with a progressive decrease in creatinine levels and reaching a concentration of 164.7 μmol/L by day 8.

A protocol for dual induction of immunosuppressive therapy based on anti-thymocyte globulin and anti-CD-25 antibodies, adopted at the Volzhsky branch of Shumakov National Medical Research Center of Transplantology and Artificial Organs, was used during KTx [13]. From day 4, the patient was transferred to standard triple immunosuppressive therapy (methylprednisolone, tacrolimus, mycophenolic acid). Colchicine was discontinued. On day 16 after KTx, anti-interleukin-1β monoclonal antibody (canakinumab) was administered in a dosage of 150 mg. The patient was discharged on day 18 after KTx (creatinine level 121 μmol/L and CRP 8.8 mg/L). Kidney function (creatinine level) at 8 months after transplantation is shown in Fig. 1.

Subsequently, the patient continued to receive standard triple immunosuppressive therapy, and monotherapy with anti-interleukin-1β monoclonal antibody (canakinumab) was continued in 150 mg dosage once

every 4 weeks to control the autoinflammatory process characteristic of the recurrent disease. The dynamics of the inflammatory marker are presented in Fig. 2.

The use of anti-interleukin-1β monoclonal antibodies allows to control the subclinical activity of inflammatory markers in FMF and significantly slow down progression of this autoinflammatory disorder [13].

CONCLUSION

The clinical case presented by us demonstrates the possibility of treating an FMF patient complicated by secondary amyloidosis using modern genetically engineered drugs. The use of biological therapy allows to perform KTx in patients with FMF, obtaining outcomes comparable to those in other groups of patients

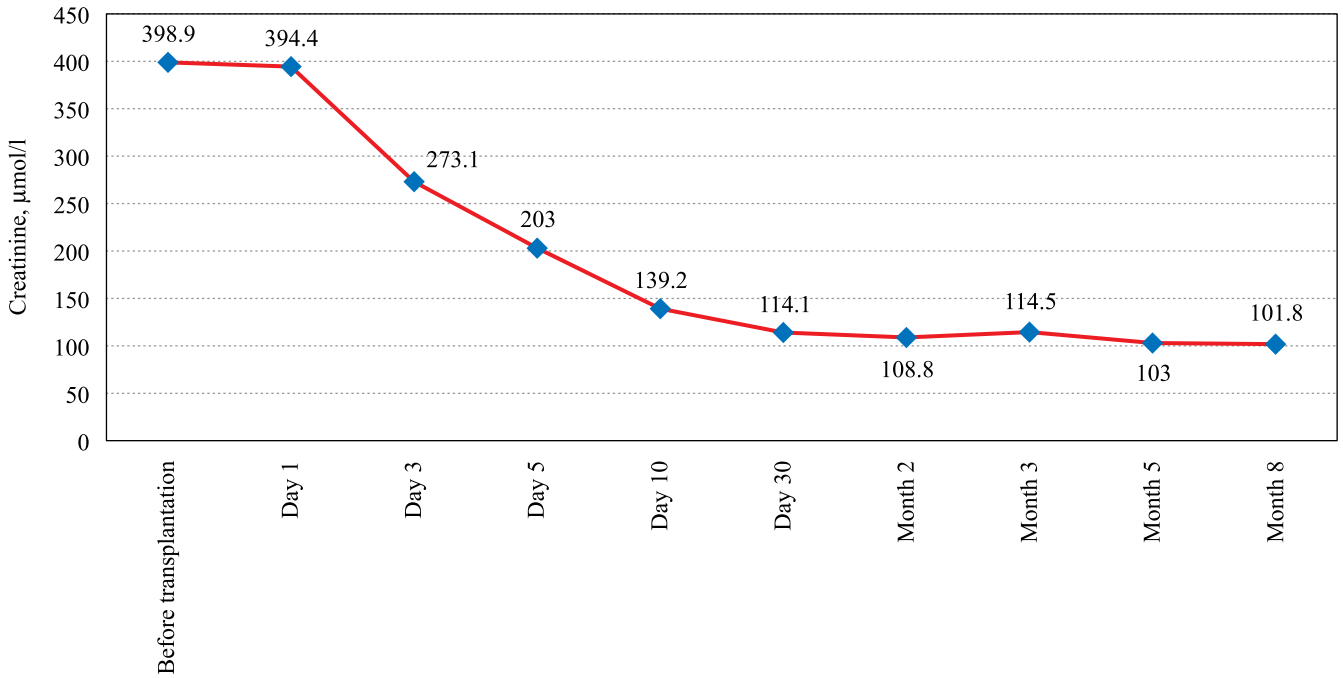

Fig. 1. Dynamics of creatinine levels in patient C. for 8 months post-kidney transplant

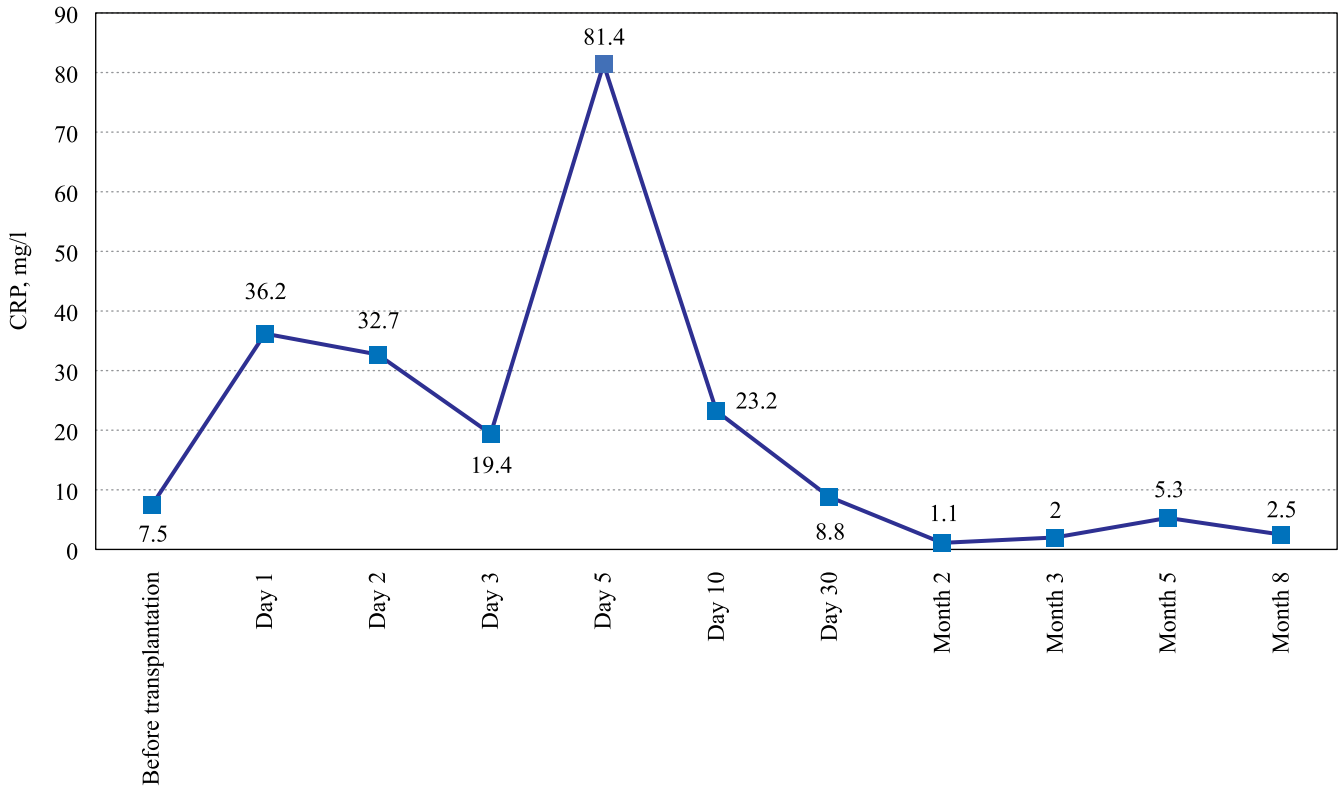

Fig. 2. Dynamics of C-reactive protein for 8 months post-transplant

with chronic kidney disease. Further monitoring of the patient's condition will make things clearer on kidney graft function and effectiveness of therapy.

The authors declare no conflict of interest.

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PERSONALIZED DOSING PROTOCOL FOR EXTENDED-RELEASE TACROLIMUS IN KIDNEY TRANSPLANT RECIPIENTS IN THE EARLY POSTOPERATIVE PERIOD

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Objective: to develop a personalized algorithm for extended-release tacrolimus in kidney recipients and to analyze its early outcomes in comparison with a retrospective control group. **Materials and methods.** The first (I) control group "Standard Protocol" included 228 patients operated on at Botkin City Clinical Hospital from June 2018 to November 2021; tacrolimus was administered postoperatively in a starting standard dosage of 0.2 mg/kg. The second group (II) consisted of 75 patients operated from December 2021 to November 2022, whose postoperative treatment involved a personalized extended-release tacrolimus dosing protocol. Induction immunosuppression was similar in both groups. The target tacrolimus level in the early postoperative period was considered to be 10–12 ng/ml for all patients. The comparison criteria included incidence of Over-immunosuppression (tacrolimus C_0 >15 ng/ml), incidence of acute rejection and infectious complications in the first month after surgery, incidence and duration of delayed graft function (DGF), and length of stay at the hospital. **Results.** Over-immunosuppression was statistically significantly lower in the personalized protocol group, with 36.7% in group I and 87.5% in group II ($p < 0.001$). There was also a lower incidence of early infectious complications in group II: 5.4% vs. 13.2%, however, without reaching a level of statistical significance ($p = 0.088$). DGF incidence in group I and group II were 25.4% (58/228) and 22.7% (17/75), respectively. The length of stay at the hospital in group II was also statistically significantly lower: 13 versus 19 bed days ($p = 0.033$). In both subgroups, no patient developed acute rejection in the first month after surgery ($p = 1$). **Conclusion.** The personalized dosing protocol that was developed for extended-release tacrolimus in kidney recipients achieves the target levels of the drug recommended for the early postoperative period with low risk of under-immunosuppression and associated acute graft rejection, with a significantly lower incidence of over-immunosuppression.

Keywords: tacrolimus, kidney transplantation, immunosuppressive therapy, DGF.

INTRODUCTION

Kidney transplantation (KTx) is currently regarded as the optimal treatment method for renal replacement therapy for patients without absolute contraindications. The discovery and introduction of calcineurin inhibitors (CNIs) into immunosuppression regimens was the breakthrough in clinical transplantology that made KTx the gold standard treatment for end-stage kidney disease. Introduction of cyclosporine A (CsA) in the early 1980s saw the one-year survival rate of renal transplants rise from 60% to over 80%. Tacrolimus (Tac), on the other hand, as an alternative to cyclosporine, was introduced into clinical practice in the early 1990s, and to date, its advantage over cyclosporine has been proven by many authors. Tac is much more effective in preventing acute rejection and it generally has comparable side effects with CsA [1–2]. That is why since 2009, the KDIGO guidelines have proposed Tac as the basis for most immunosuppressive maintenance therapy regimens [3].

Despite the proven superiority of Tac, its side effects not only worsen renal graft function gradually, but also cause serious diseases such as diabetes mellitus and arterial hypertension in the late post-transplant period. In the early post-transplant period, when the recommended doses and concentrations of the drug are highest, acute nephrotoxicity is the most common and undesirable effect in renal transplant recipients [4–5]. It is based on changes in hemodynamics at the microcirculatory level, endothelial cell dysfunction, tubular damage, development of thrombotic microangiopathy and impaired ion exchange, leading to reduced glomerular filtration rate (GFR) and increased plasma creatinine levels. In addition to increased vasoconstrictor synthesis, CNIs cause endothelial dysfunction by reducing NO synthesis. There is also an increase in the formation of free radicals and

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superoxides, probably due to hypoxia associated with efferent arteriolar vasoconstriction [6].

One of the most common early postoperative complications is delayed graft function (DGF). It is commonly defined as the need for hemodialysis within 7 days following a KTx; its pathogenesis is based on kidney graft damage during cold preservation and reperfusion [7]. Development of CNI-induced acute nephrotoxicity with the need for hemodialysis in the first week after KTx, according to the classical definition, may be the cause of DGF [8]. This side effect of Tac is usually dose-dependent and completely reversible after correction; however, performing hemodialysis sessions in the early postoperative period may be a risk factor for more serious complications.

In our previous study on the search for DGF risk factors, DGF was found to have a statistically significant association with increased incidence of early postoperative complications and decreased long-term graft survival [9]. According to multivariate analysis, increased Tac zero levels in the first 4 days >23 ng/ml was statistically significant and was an independent risk factor for DGF ($p = 0.025$). This prompted a revision of the then-existing protocol for immunosuppressive therapy after kidney transplantation in our clinic and the development of a personalized dosing algorithm for extended-release Tac. The purpose of this study was to analyze the initial outcomes of the use of this protocol in comparison with a retrospective control group.

MATERIALS AND METHODS

From June 2018 through November 2022, 337 isolated kidney transplants from deceased donors were performed at Botkin City Clinical Hospital. Organ explantation from the donor, cold preservation, and surgical technique were performed according to the standard protocols of the National Clinical Guidelines. For immunosuppression in the early postoperative period, a triple regimen consisting of extended-release Tac, mycophenolic acid derivatives and methylprednisolone was used as standard. Basiliximab (20 mg) was given as induction therapy intraoperatively and on day 4; methylprednisolone (10 mg/kg) was administered intraoperatively and on days 3 and 5 intravenously. The starting Tac dose that the patient received before surgery was determined at 0.2 mg/kg from 2018 to 2021, and thereafter (2021–2022) according to the personalized protocol developed. The target Tac level in the early postoperative period was considered to be 10–12 ng/ml for all patients.

Group characteristics

The first control group of the "Standard IST Protocol" included 228 patients operated on at Botkin City Clinical Hospital from June 2018 to November 2021. There were 83 (36.5%) women and 145 (63.5%) men in this group. The mean age was 47 ± 11 (IQR: 39–55) years, mean recipient BMI was 25.5 (IQR: 23.0–29.0) kg/m^2, residual diuresis was 300 (IQR: 100–600) ml, donor age was 49 (IQR: 44–54) years, donor BMI was 26.0 (IQR: 24.2–30.0) kg/m^2, cold preservation time was 625 (IQR: 515–740) minutes, secondary warm ischemia time was 40 (IQR: 30–50) minutes, and intraoperative blood loss was 100 (IQR: 50–150) ml. The most common causes of end-stage renal disease in group 1 were chronic glomerulonephritis (61%), chronic pyelonephritis (9%), polycystic disease (9%), diabetic nephropathy (9%), chronic tubulointerstitial nephritis (5%), etc. In 225/228 cases, the kidney graft was obtained from brain-dead donors, and in 3 cases, the graft was procured from a donor with effective circulatory arrest.

The second group consisted of 75 patients operated on at Botkin City Clinical Hospital between December 2021 and November 2022, whose postoperative treatment involved a personalized dosing protocol for extended-release Tac developed at the Department of Organ and Tissue Transplantation, Botkin City Clinical Hospital. There were 33 (36.5%) women and 42 (63.5%) men in this group. Median age was 46 (IQR: 38–54) years, median recipient BMI was 26.0 (IQR: 24.0–27.3) kg/m^2, residual diuresis was 300 (IQR: 0–700) ml, donor age was 51 (IQR: 48–52) years, donor BMI was 31.5 (IQR: 27.4–34.0) kg/m^2, cold preservation time was 710 (IQR: 640–780) minutes, secondary warm ischemia time was 40 (IQR: 30–47) minutes, and intraoperative blood loss was 100 (IQR: 100–200) ml. The most common causes of end-stage renal disease in group II were chronic glomerulonephritis (54%), chronic pyelonephritis (9%), polycystic disease (8%), diabetic nephropathy (15%), chronic tubulointerstitial nephritis (5%), etc. In 73/75 cases, the renal transplant was obtained from brain-dead donors, and in 2 cases from donors with effective circulatory arrest. Primary graft nonfunction (PNF), a need for revision and graftectomy within the first week after transplantation, and the use of hypothermic oxygenated machine perfusion during graft preservation were the exclusion criteria. The characteristics of the groups are presented in Table 1.

In creating a personalized dosing protocol for extended-release Tac at the first stage, we determined the really necessary doses of the drug per kg of each patient's weight, which they received 3 weeks after surgery (at the time of discharge). Calculation of the optimal dose included group 1 patients who received a triple immunosuppressive regimen (extended-release Tac + mycophenolic acid preparations + methylprednisolone), provided they achieved the target concentration consistently required for the appropriate postoperative period. After that, for 204 patients selected from the retrospective group, we evaluated the significance of the influence of several quantitative and qualitative characteristics of the recipient on the required Tac dose: volume of residual diuresis, sex, age, weight, body mass index, history of kidney

transplantation, etiology of chronic kidney disease, product and sum of recipient age and weight.

After developing and implementing a personalized immunosuppressive therapy protocol into clinical practice, we evaluated its safety and efficacy in a comparative study. The following parameters were analyzed: incidence of over-immunosuppression (Tac pre-dose concentration C_0 >15 ng/mL), incidence of acute rejection and infectious complications in the first month after surgery, incidence of DGF and its duration, and length of stay at the hospital. DGF was defined as the requirement for hemodialysis within the first 7 days following renal transplantation.

Statistical analysis

Statistical processing and data analysis were performed using SPSS Statistics for Microsoft Windows version 26 (USA). Student's t-test or Welch's t-test was used to compare two groups of quantitative indicators with normal distribution (depending on equality of variance). For distributions other than normal, the Mann–Whitney U test was used to compare two groups of quantitative data, while the Kruskal–Wallis H test was used to compare three or more groups. Qualitative indicators were compared using Pearson's chi-squared test or Fisher's exact test, with the determination of the odds ratio (OR) and closeness of correlation between the characteristics under study. Correlation analysis was performed by the Spearman method with determination of rank correlation coefficient ρ and closeness of correlation according to Cheddock's scale. The dependence of changes in quantitative indicators on each other was assessed in a linear regression model. Pseudorandomization was performed in SPSS Statistics v. 26 using the PSM method with a fit tolerance of 0.1. Statistically significant differences were considered at $p < 0.05$, the trend towards statistical significance was defined as $p < 0.1$.

RESULTS

Development of a personalized dosing protocol for extended-release tacrolimus

Among the 204 patients selected from the retrospective group, 34 (17%) had a calculated Tac dose of less than 0.1 mg/kg at discharge, 121 (59%) were in the range of 0.1–0.19 mg/kg, and only 49 (24%) patients needed a dose of 0.2 mg/kg or more. In the analysis of factors influencing the required drug dose 3 weeks after KTx, such parameters as age ($p = 0.012$), weight and BMI ($p = 0.009$ and $p = 0.021$), product and sum of recipient age and weight ($p = 0.005$ and $p = 0.0023$), achieved statistical significance. At the same time, the inverse correlation of the highest closeness on the Cheddock's scale was demonstrated precisely for the sum of the patient's age and weight ($\rho = –0.706$). Analysis of the effect of various recipient characteristics on the required dose of extended-release Tac at the time of discharge is shown in Table 2.

Table 1

Characteristics of the study groups

	Group I Standard ISx protocol (2018–2021) n = 228	Group II Modified ISx protocol (2021–2022) n = 75	p
Recipient age (years)	47 (IQR: 39–55)	46 (IQR: 38–54)	0.867
Recipient BMI (kg/m^2)	25.5 (IQR: 23.0–29.0)	26.0 (IQR: 24.0–27.3)	0.65
Residual diuresis (ml)	300 (IQR: 100–600)	300 (IQR: 0–700)	0.756
Donor age (years)	49 (IQR: 44–54)	51 (IQR: 48–52)	0.15
Donor BMI (kg/m^2)	26.0 (IQR: 24.2–30.0)	31.5 (IQR: 27.4–34.0)	0.03
Cold preservation time (min)	625 (IQR: 515–740)	710 (IQR: 640–780)	0.015
Secondary warm ischemia time (min)	40 (IQR: 30–50)	40 (IQR: 30–47)	0.83
Intraoperative blood loss (ml)	100 (IQR: 50–150)	100 (IQR: 100–200)	0.73
Donor type: – *SD* – *ECD* – *DCD*	 115 (50.4%) 109 (47.8%) 4 (1.8%)	 38 (50.6%) 34 (45.3%) 3 (4.1%)	>0.05
Etiology of CKD: – *Chronic glomerulonephritis* – *Chronic pyelonephritis* – *Diabetic nephropathy* – *Polycystic kidney disease* – *crTIN* – *Others*	 139 (61%) 21 (9%) 21 (9%) 21 (9%) 11 (5%) 15 (7%)	 40 (54%) 6 (8%) 11 (15%) 6 (8%) 8 (9%) 4 (6%)	>0.05

Note: SD, standard donor; ECD, expanded criteria donor; DCD, donor after cardiac death; CrTIN, chronic tubulointerstitial nephritis.

Based on the identified statistically significant and closest inverse correlation between the required Tac dose per kg of body weight and the sum of the recipient's age and weight, a predictive linear regression model was built. The observed dependence is described by the equation:

$$Y_{dose} = 0.285 - 0.001 \times X_{age + weight},$$

where Y_{dose} is the extended-release Tac dose and $X_{age + weight}$ is the sum of the recipient's age and weight. A graphical representation of the linear regression model is shown in Fig. 1.

The significance level was $p < 0.001$. Based on the value of the coefficient of determination (R^2), the factors included in the model determined 28.5% of variance. To categorize patients into groups according to the extended-release Tac dose administered, cut-off points were determined by the sum of the recipient's age and weight by a combined analysis of median, quartiles (25% and 75%), most sensitive and specific cutoff points from ROC analysis, and applicability in clinical practice. Thus, patients whose sum of age and weight was less than 105 were given extended-release Tac at 0.2 mg/kg of recipient body weight; those with a sum of 105 to 134 got 0.15 mg/kg, and patients with a sum of more than 134 got 0.1 mg/kg. The personalized dosing protocol for extended-release Tac is shown in Fig. 2.

Comparative analysis of the safety and efficacy of the personalized dosing protocol for extended-release tacrolimus with respect to the standard regimen

The incidence of over-immunosuppression (Tac predose concentration C_0 >15 ng/mL) in Group I was 87.5% of patients (199/228), in the personalized immunosuppression (ISx) protocol group this rate was 34.7% (26/75) ($p < 0.001$). No episodes of under-immunosuppression were reported in any case of the personalized Tac dosing

Table 2

Influence of various indicators on the required extended-release tacrolimus dose

Indicator	p value	Spearman's rank correlation coefficient ρ (for quantitative indicators)	Strength of correlation according to the Cheddock's scale
Recipient residual diuresis	0.73	–	–
Recipient gender	0.321	–	–
Recipient age	**0.012**	–0.352	Moderate
CKD etiology	0.41	–	–
Repeat kidney transplantation	0.512	–	–
Recipient weight	**0.009**	–0.413	Moderate
Recipient BMI	**0.021**	–0.248	Weak
Product of recipient age and weight	**0.005**	–0.678	Moderate
Sum of recipient age and weight	**0.0023**	–0.706	Strong

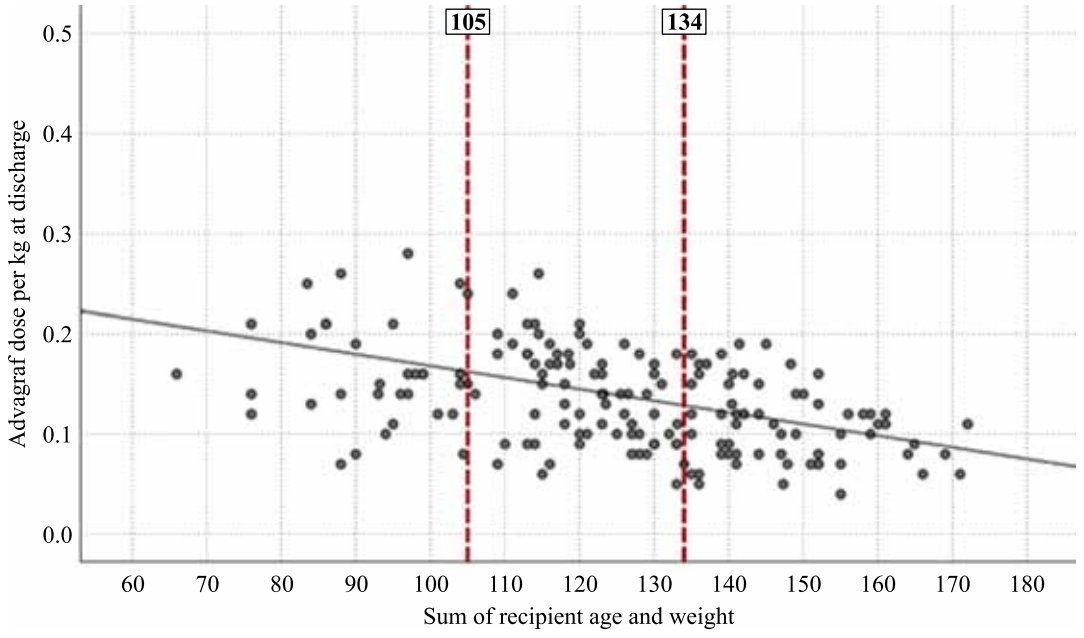

Fig. 1. Dependence of extended-release tacrolimus dose on the sum of recipient age and weight (linear regression)

protocol, and in both subgroups, no patient developed acute rejection in the first month after surgery (p = 1). In group II, there was also a decreased incidence of early infectious complications: 5.4% versus 13.2%, but without reaching the level of statistical significance (p = 0.088). The incidence of delayed renal graft function was 25.4% (58/228) in retrospective group I and 22.7% (17/75) in group II. There were no statistically significant differences in this indicator between the groups (p = 0.629). The groups were also comparable in terms of DGF duration and length of stay at the hospital (p = 0.238 and p = 0.521, respectively).

However, given the presence of statistically significant differences between the groups in terms of donor BMI and static cold preservation time (p = 0.03 and p = 0.015, respectively), we performed pseudorandomization by PSM method for these factors. This resulted in new groups comparable in terms of the above parameters (p = 0.745 and p = 0.612, respectively), with 38 patients in each. DGF incidence in the group using the standard ISx protocol after PSM was 12/38 (31.6%), while in group II it was 4/38 (10.8%). The differences were statistically significant (p = 0.047) and there was a moderate strength relationship between the traits (V = 0.258). The odds of developing DGF with the personalized ISx protocol were 3.9 times lower than in the control group (95% CI: 1.1–13.6). The median duration of DGF in the personalized protocol group was 8 (IQR: 7–11) days, and 15 (IQR: 9–15) days in the control group (p = 0.016). The length of hospital stay in the personalized protocol group was also statistically significantly lower: 13 (IQR: 8–19) versus 19 (IQR: 15–24) bed-days (p = 0.033). The results of the efficacy and safety study of the developed personalized protocol compared with the standard protocol for extended-release Tac before and after pseudorandomization are presented in Table 3.

Fig. 2. Personalized protocol for extended-release tacrolimus based on the sum of recipient age and weight

Table 3

Comparative analysis of the outcomes of standard and personalized dosing protocols for extended-release tacrolimus after kidney transplantation

Indicator	Before PSM			After PSM		
	Group I Standard ISx protocol n = 228	Group II Personalized ISx protocol n = 75	p	Group I Standard ISx protocol n = 38	Group II Personalized ISx protocol n = 38	p
Donor BMI (kg/m^2)	26.0 (IQR: 24.2–30.0)	31.5 (IQR: 27.4–34.0)	**0.03**	28.0 (IQR: 22.5–30.0)	29.5 (IQR: 21.5–31.0)	0.745
Cold preservation time (min)	625 (IQR: 515–740)	710 (IQR: 640–780)	**0.015**	650 (IQR: 620–660)	660 (IQR: 620–670)	0.612
Over-immunosuppression (tacrolimus pre-dose level (C_0) >15 ng/ml)	199 (87.5%)	26 (34.7%)	**<0.001**	34 (89.5%)	15 (39.5%)	**<0.001**
Acute graft rejection in the first month after kidney allotransplantation	0	0	1	0	0	1
Infectious complications in the first month after kidney allotransplantation	29 (12.7%)	4 (5.3%)	0.088	6 (15.8%)	1 (2.6%)	0.108
DGF	58 (25.4%)	17 (22.7%)	0.629	12 (31.6%)	4 (10.5%)	**0.047**
Median duration of DGF (days)	13 (IQR: 8–16)	14 (IQR: 6–16)	0.238	15 (IQR: 9–15)	8 (IQR: 7–11)	**0.016**
Median duration of hospitalization (13 bed days)	16 (IQR: 12–19)	15 (IQR: 10–21)	0.512	19 (IQR: 15–24)	13 (IQR: 8–19)	**0.033**

DISCUSSION

Tacrolimus-associated nephrotoxicity is one of the most important problems of immunosuppressive therapy in kidney transplant recipients. It prompts researchers and clinicians to constantly search for methods to reduce the risk of its development. Acute nephrotoxicity, which develops in the first week after transplantation against the background of increased CNI levels, may be one of the main reasons for DGF. On the other hand, immunological response is maximal during the immediate post-transplant period, which requires higher doses and maintaining higher CNI concentrations than in the long-term period. Therefore, the main challenge in prescribing immunosuppressive therapy during this period is to maintain a balance between adequate prevention of acute rejection and minimization of the risk of side effects.

To solve this problem, many authors propose various strategies to reduce CNI burden in the early post-transplant period. One of them is the administration of low Tac doses (with a target concentration of 3–8 ng/mL) in combination with mTOR inhibitors immediately after transplantation [10–12]. In our opinion, inclusion of these drugs in immunosuppressive regimens in the long-term postoperative period to reduce the effect of CNI-related nephrotoxicity in some cases is certainly justified. However, in the first days after transplantation, the use of mTOR inhibitors may be associated with increased risk of wound infection. Another strategy may be to delay Tac administration, when it is administered on day 4–7 after transplantation. A number of national [13] and foreign [14] studies have found that delayed Tac administration leads to improved long-term survival of kidney transplants, but no statistically significant effect on improvement of initial graft function is demonstrated. At the same time, according to some authors, delayed Tac administration for 4–7 days does not increase the incidence of acute rejection [13–14], while according to other authors, the risk of rejection significantly increases [15]. Thus, the safety and efficacy of this strategy on preventing acute nephrotoxicity in the early postoperative period, in our opinion, is questionable and requires more investigation.

As mentioned above, the first weeks after transplantation are the most critical period, since the recipient's immune response to the allograft is maximally expressed. Excessive minimization of immunosuppression can lead to an unacceptable risk of steroid-resistant rejection and early graft loss. It should be noted that in our study, none of the kidney transplant recipients had acute rejection in the early postoperative period. This can most likely be attributed to the fact that the use of the standard dosing protocol for prolonged-release tacrolimus led to a "balance shift" towards over-immunosuppression in 87.5% of cases, which also worsened the immediate and long-term outcome of KTx. While developing the personalized dosing protocol, we found that a starting Tac dose of 0.2 mg/kg, which was routinely administered to all patients immediately before surgery, was actually needed in less than 25% of patients. Half of the patients required a dose in the range of 0.1–0.2 mg/kg, and 17% required less than 0.1 mg/kg. Our study revealed a statistically significant pattern: the higher the age and weight of the recipient, the lower the Tac dose required ($\rho = -0.706$, $p = 0.0023$). These indicators may be somehow related to the intensity of metabolic processes in the liver, where tacrolimus is metabolized.

A more detailed study of the genetic features of the cytochrome p450 enzyme system could probably help to determine a more accurate starting dose of the drug. Both in the national and world literature, there are works demonstrating the effectiveness of CYP3A5 polymorphism genotyping in transplant candidates for the most accurate selection of Tac dosage after transplantation [16–19]. According to Shuker (2016) and others, CYP3A5-expressing transplant candidates require a dose that is approximately 50% higher than most [20–21] to reach the target concentration. At the same time, despite the promise of determining the CYP3A5 genotype in a patient before KTx for subsequent calculation of the starting Tac dose, this protocol, at present, cannot become the standard for most transplant centers. The medical and cost-effectiveness of this strategy remains to be substantiated and proven by further studies.

Implementation of the developed personalized dosing algorithm for prolonged-release tacrolimus in clinical practice led to a significant decrease in the frequency of over-immunosuppression in the early postoperative period by almost 2.5 times ($p < 0.001$), but episodes of increased Tac concentration (>15 ng/ml) were still recorded in a significant proportion of recipients. Obviously, the recipient's age and weight are not the only factors that can influence the drug dose required to achieve the target concentration, and in 34.7% of cases the administered dose was slightly higher than that actually needed. Nevertheless, we found a significant improvement in early graft function, which was expressed by statistically significant reduction in the frequency and duration of DGF ($p = 0.047$ and $p = 0.016$), as well as the length of hospital stay ($p = 0.033$) in the groups comparable in terms of main characteristics. Decreased CNI burden also resulted in a reduced risk of early infectious complications, but only with a trend toward statistical significance ($p = 0.088$). This is probably due to insufficient number of cases. At the same time, it is important to note that the use of the new protocol did not lead to increased incidence of under-immunosuppression and acute rejection. We also found a significant improvement in early graft function, which was expressed by a statistically significant decrease in the incidence and duration of DGF ($p = 0.047$ and $p = 0.016$), as well as the length of stay at the hospital ($p = 0.033$).

Thus, administration of a starting dose of extended-release Tac according to our protocol based on the patient's individual characteristics – age and body weight – allows both to ensure adequate immunosuppression and minimize the risk of rejection, and to reduce the burden of acute nephrotoxicity and other side effects of calcineurin inhibitors in the early post-transplant period.

CONCLUSIONS

1. The developed personalized dosing protocol for extended-release tacrolimus in patients after KTx allows achieving the recommended target drug concentrations for the early postoperative period with low risk of under-immunosuppression and associated acute graft rejection with significantly lower incidence of over-immunosuppression ($p < 0.001$).
2. Introduction of the personalized dosing protocol in the clinical practice of kidney transplantation allowed to significantly reduce the incidence of DGF from 31.6% to 10.5% ($p = 0.047$) in the groups in terms of the main risk factors of this complication.

The authors declare no conflict of interest.

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ASSESSMENT OF THE QUALITY OF LIFE OF ORGAN RECIPIENTS BASED ON THE RESULTS OF THE FIRST RUSSIAN TRANSPLANT GAMES

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Objective: to evaluate the role of physical activity (at sports games) in improving the quality of life of organ recipients. **Materials and methods.** We examined 42 adult lung, heart, kidney and liver recipients, and patients undergoing renal replacement therapy (mean age 42.6 ± 12.09 years) – participants of the First Russian Transplant Games. The results were analyzed. Quality of life of the recipients was assessed using the nonspecific SF-36 questionnaire. **Results.** After solid organ transplantation, the interviewed recipients answered that they try to lead an active lifestyle, to engage in accessible physical activities, and to participate in sports events intended for organ recipients. Assessment of the quality of life according to the SF-36 questionnaire showed that all the participants had high scores in terms of the physical and psychological component, which is associated with regular physical training and sports. **Conclusion.** Physical exercise and active participation in sports activities are an important component in the socialization and rehabilitation of organ recipients. These two factors also improve the psychological and physical components of the quality of life of the recipients.

Keywords: transplantation, physical rehabilitation, physical exercise, dialysis, quality of life, exercise therapy, periods of medical and social rehabilitation, sports.

INTRODUCTION

Physical activity plays an important role in shaping a person's physical and psychological health. An active lifestyle reduces the risk of developing socially significant diseases like type II diabetes, cancer, and hypertension. It has a beneficial effect on the quality of life of both healthy people and organ recipients, and patients on renal replacement therapy [1–5].

Sports competitions, games, and festivals are organized among organ transplant recipients to promote physical fitness and sports, increase public awareness and popularize the possibilities of transplantation and organ donation [6]. People who have undergone organ transplantation can once again demonstrate high physical and mental health after rehabilitation [7].

After organ transplantation, it is possible to lead an active lifestyle, engage in physical training and sports, as evidenced by various sports events among people with organ transplantation. For example, the World Transplant Games have been held since 1978, and patients from Russia also take part in them [8, 9].

In Russia, a public charity event titled "People for People's Sake" (Fig. 1) has been held from 2011 to 2016. The event included a football match among patients, doctors, and public figures in order to attract society to the problem of organ donation in Russia [10]. But since then, no systematic sports competitions for people with transplanted organs have been held in our country.

Thanks to the organizers of the project – Shumakov National Medical Research Center of Transplantology and Artificial Organs, NEFRO-LIGA (an interregional public organization of nephrology patients), Pirogov Russian National Research Medical University and Svaya Atmosphera (an interregional charitable public organization) – the First Russian Transplant Games were held in Moscow from July 29 to 31, 2022 with the participation of over 70 recipients from all regions of the country. Contestants competed in running, football, basketball, volleyball, tennis, badminton, swimming, darts, Nordic walking, and chess [11].

In between competitions, master classes in yoga, bowling, and dancing were held for the participants. Patients not only competed in various sports disciplines, but also acted as coaches. For example, training in Zumba fitness dance was held by a teacher, who is a renal replacement therapy patient.

All participants noted that such sporting events helped them to unite and meet acquaintances with whom they communicated only online, find new friends and support among patients, doctors, volunteers and orga-

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nizers, try themselves in different sports and activities, visit a new region or country, become an example for people who are afraid to undergo transplantation. Life after transplantation can again be full, rich with aspirations and joys of victories, including victories over oneself (Fig. 2).

The quality of life of organ recipients depends on both the course of the disease itself and the type of renal replacement therapy. An important criterion for assessing morbidity and mortality, as well as the health of the population, is to determine the relationship between the quality of life and the health of patients [12, 13]. With the help of questionnaires that present physical, emotional, mental, social and behavioral components, it is possible to identify and standardize the indicators of the quality of life of those under study [14]. It is the analysis of a patient's quality of life that provides a complete picture of the psychosocial and physical impact of organ transplantation and renal replacement therapy.

MATERIALS AND METHODS

Seventy adult lung, heart, kidney and liver recipients, as well as patients undergoing renal replacement therapy from 18 regions of the Russian Federation (Volgograd Oblast, Sverdlovsk Oblast, Republic of Tatarstan, Kaliningrad, Omsk Oblast, Moscow and Moscow Oblast, Republic of Buryatia and others) took part in the sport competition. Before the competition, all participants underwent a professional examination by a doctor, tests and a doctor's report confirming satisfactory graft function were provided. No adverse reactions were noted during the competition. A questionnaire was administered to 42 contestants at the First Russian Transplant Games who underwent organ transplantation and patients on renal replacement therapy. At the time of the competition, the recipients had different postoperative periods: minimally, 6 months after transplantation and maximally, 18–19 years after transplantation; durations of renal replacement therapy were minimally, 3 years and maximally,

Fig. 1. Public charity event "People for People's Sake"

Fig. 2. Group photo of winners of the First Transplant Games (2022)

19 years. Participants, at will, were offered a questionnaire developed by us and a quality-of-life testing using the SF-36 questionnaire.

Quality of life (physical and mental component) was assessed according to 8 scales, which had a scoring system (from 0–100 points): physical functioning (PF), role-physical functioning (RF), bodily pain (BP), general health (GH), mental health (MH), role-emotional functioning (RE), social functioning (SF), and vitality (VT).

RESULTS

Questionnaires were analyzed for 42 participants of different ages: from 19 to 70 years old (mean age, 42.6 ± 12.09), who underwent transplantation of various organs and patients on renal replacement therapy (Table).

Table

Distribution of contestants by sex, age and transplanted organ (n = 42)

Age, gender		Transplanted organ						
		heart	liver	lungs	kidney	kidney retransplantation	Renal replacement therapy	Total
19	M				1			1
	F				1			1
20–29	M	1			1			2
	F		1		1			2
30–39	M	2			4		2	8
	F			1	1	2	2	6
40–49	M	1			2		1	4
	F	2	1	1	3		2	9
50–59	M				1			1
	F				3	1		4
60–69	M		1					1
	F				2			2
70	M	1						1
	F							0
Total		7	3	2	20	3	7	42

Seven heart recipients (5 men and 2 women), 3 liver recipients (1 man and 2 women), 2 lung recipients (women), 23 kidney recipients (9 men and 14 women), including 3 women after repeat kidney transplantation, and 7 patients (3 men and 4 women) undergoing renal replacement therapy (hemodialysis) participated in the survey.

Patients with the most active lifestyle, both before the disease and after surgical treatment, participated in the sports games. Only 6 out of 42 participants surveyed were not engaged in physical training before the disease, the rest led an active lifestyle, played football, mini-football, wrestling, athletics, sports dancing, swimming, volleyball and other amateur sports. Seven of them were engaged in professional sports and had different sports titles: the 2nd category for youth in cross-country skiing and track and field athletics, the 2nd category for adult volleyball, candidate master of sports in acrobatics and biathlon, master of sports in cycling, champion of Tatarstan in mini football in 2002.

After solid organ transplantation, all patients under study tried to lead an active lifestyle, engage in moderate physical activity, and participate in sports events for patients with transplanted organs. For instance, one of the recipients participated in the World Transplant Games in 2017, 2018, and 2019; a group of patients in 2022 took part in the country's traditional most popular 10- and 42-km races, the Moscow Marathon (Fig. 3).

Donor organ recipients are at risk of developing many diseases. Therefore, there was a special focus on identifying risk factors for cardiovascular diseases, which are one of the leading causes of mortality among this category of patients [12].

Fig. 3. Group photo of participants (patients and doctors) of the Moscow Marathon (2022)

None of the participants were habitual smokers, only 2 people admitted to episodic disorders.

The patients under study adhered to the doctor's dietary recommendations and controlled their body weight. Figs. 4 and 5 show the characteristics of the patients' state according to the body mass index (BMI) in men and women.

Thus, a normal BMI was body mass index in 68.4% (n = 13) of men and 39.1% (n = 9) of women, overweight was diagnosed in 26.3% (n = 5) of men and 20.1% (n = 6) of women, 5.3% (n = 1) of men and 17.4% (n = 4) of women had malnutrition, and hypotrophy degree 1 was found in 17.4% (n = 4) of women.

Our study revealed certain regularities: assessment of the quality of life on the SF-36 questionnaire showed that all the participants had high scores on the scales of general health, vitality, and mental health. High scores in the scales of physical functioning (85.5%), role-physical functioning (78.9%) and role-emotional functioning (84.5%), which is associated with regular exercise and sports (Fig. 6).

All participants indicated that organ recipients need sports activities, which is important. 100% of surveyed patients answered positively to the following questions in the questionnaire: "Do you think you need to engage in sports and physical exercise?", "Is it necessary to develop sports community among organ recipients?", "Do you need sports games for organ recipients in Russia?", "Would you like to participate in the World Transplant Games?".

CONCLUSION

The study showed that there were high indicators of physical and mental condition of recipients participating in the First Russian Transplant Games. In the course of the study, each participant underwent a professional examination by a sports medicine physician, and recommendations on physical activity were given to the recipients – participants in the competitions.

Thus, physical training and sports activities can improve the quality of life in the psychological and physical areas, and reduce cardiovascular disease risk factors in solid organ recipients.

Based on the survey results, it was decided to hold the Second Russian Transplant Games, which will take place in Moscow on July 27–30, 2023.

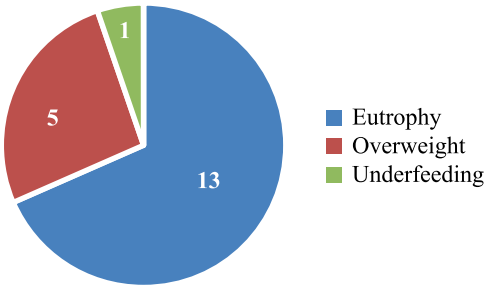

Fig. 4. Characteristics of nutritional status by BMI in men (n = 19)

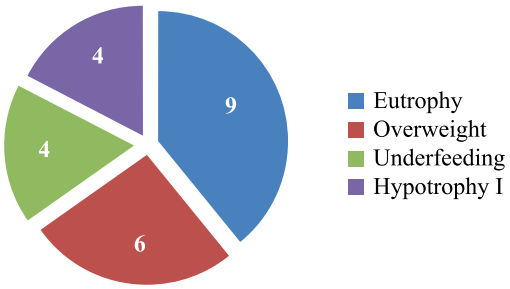

Fig. 5. Characteristics of nutritional status by BMI in women (n = 23)

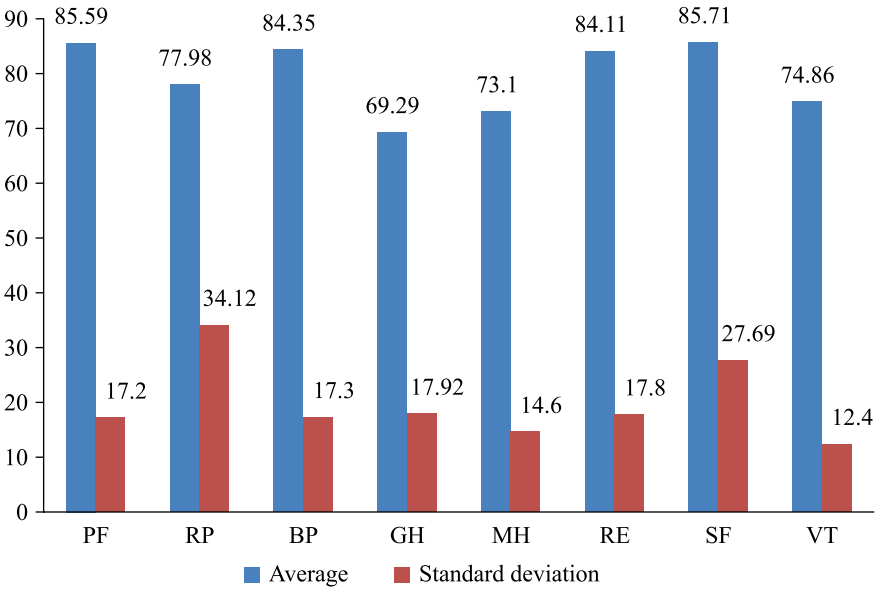

Fig. 6. Indicators of the quality of life of participants in sports games according to the SF-36 questionnaire

The authors declare no conflict of interest.

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NONSELECTIVE BETA-BLOCKERS IN PRIMARY PROPHYLAXIS OF ESOPHAGEAL VARICEAL BLEEDING IN PATIENTS WITH ASCITES WAITLISTED FOR LIVER TRANSPLANTATION

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Objective: to determine the efficacy of non-selective beta-blockers (NSBBs) in the primary prevention of bleeding esophageal varices and to assess their impact on the survival of patients with ascites enrolled in the liver transplant waiting list (LTWL). **Materials and methods.** We carried out a retrospective comparative study of cirrhotic patients with severe ascites and esophageal varices without bleeding before enrollment in the LTWL. Primary prophylaxis of variceal bleeding included the use of NSBBs (n = 97, group 1). These drugs were not used in the other patients (n = 91, group 2). **Results.** There were no significant differences between the groups in terms of clinical, laboratory and demographic parameters, MELD scores and Child–Turcotte–Pugh (CTP) classes for cirrhosis. Patient groups included in the study had no significant differences with respect to incidence of medium- and large-sized varices and incidence of severe ascites. Bleeding incidence was significantly lower in the NSBBs group than in the non-NSBBs group (52.6% and 95.6%, respectively, p = 0.0001). **Conclusion.** NSBBs constitute an efficacious therapy in primary prophylaxis of esophageal variceal bleeding, thereby saving life and preventing delisting of patients with ascites from the LTWL.

Keywords: liver transplant waiting list, ascites, bleeding, nonselective beta-blockers.

INTRODUCTION

Clinically significant portal hypertension (CSPH) causes the transition of liver cirrhosis from a compensated to a decompensated stage, characterized by severe complications like ascites, variceal bleeding, gastric variceal bleeding, hepatic encephalopathy (PE) [1–3]. Development of decompensated cirrhosis is an indication for inclusion of patients into the liver transplant waiting list (LTWL) [1]. Despite the undoubted successes of liver transplantation (LTx), characterized by an increase in the number of saved patients, Russia and the world at large are experiencing the problem of donor organ shortage, and, as a consequence, increased waiting time for liver transplantation in patients with decompensated cirrhosis [4–6].

Increased waiting time for LTx causes high risk of mortality due to CSPH progression and developing complications, which predetermines the urgent task of saving lives and preventing attrition of patients from the LTWL [1, 7]. The International Consensus on the Management of CSPH Patients (Baveno VII) recommends that patients with ascites and medium-large varices (≥5 mm) with Child–Turcotte–Pugh class C be submitted to primary prophylaxis of bleeding with either nonselective beta-blockers (NSBBs) or endoscopic variceal ligation (EVL) in order to reduce mortality. The Baveno VII guidelines recommend the use of traditional NSBBs or carvedilol for the prevention of the first bleeding episode in this category of patients, reserving a place for EVL for patients with intolerance, or with contraindications to the use of beta-blockers [1].

MATERIALS AND METHODS

Included in a retrospective comparative study conducted at the Center for Surgery and Donor Coordination, Rostov Regional Clinical Hospital, after obtaining approval from the local ethics committee, were 188 patients with decompensated cirrhosis of viral and alcoholic etiology.

The inclusion criteria were: absence of variceal bleeding before inclusion in LTWL, ascites of varying severity, alcohol abstinence confirmed by narcologist reports for at least 3 months before inclusion in LTWL in patients with alcohol-induced cirrhosis.

Exclusion criteria: patients with hepatocellular carcinoma or other malignant diseases accompanied by ascites.

The first group of patients consisted of 97 patients who underwent primary prophylaxis of variceal bleeding

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using NSBBs, the second group consisted of 91 patients who received no NSBBs for various reasons (intolerance, contraindications).

The primary endpoint of the study was to determine the efficacy of NSBBs in the primary prevention of esophageal bleeding in the compared groups.

The secondary endpoint of the study was the study of patient survival in the compared groups.

Demographic, clinical, and laboratory parameters were obtained from a continuously updated electronic database of patients who were in LTWL for 1 to 36 months awaiting LTx. In the case of a stable state, clinical and biochemical blood tests and hemostasis indicators were repeated at 3-month intervals. The following indicators were calculated: MELD-Na [8] and Child–Turcotte–Pugh (CTP) [9, 10].

Screening esophagogastroduodenoscopy (EGD test) was performed in patients with ascites to detect varicose veins with high risk of bleeding (medium- and large-sized nodules) according to the recommendations of Baveno VI, Baveno VII (1, 11) and the World Gastroenterology Organisation (WGO) [12]. The severity of ascites was determined according to the International Ascites Club expert criteria [13].

Abdominal ultrasound was performed during initial examination of patients and every 6 months after LTx.

Group 1 patients received carvedilol (n = 46), propranolol (n = 36), and nadolol (n = 15). The initial carvedilol dose was 6.25 mg/day and the maximum dose was 25 mg/day; propranolol 40 mg/day and 240 mg/day, respectively. The initiating nadolol dose was 40 mg/day and the maximum dose was 80 mg/day. Administration of NSBBs was accompanied by monitoring of heart rate and blood pressure; the drug dose was adjusted whenever these parameters decreased. Patients in both groups received diuretics; paracentesis was performed if ascites was resistant to diuretics. Patients with HBV and HCV-associated cirrhosis received antiviral therapy with nucleoside analogues and a combination of direct-acting antivirals, respectively.

Data obtained was analyzed using the statistical program IBM SPSS Statistics (version 23). The Kolmogorov–Smirnov test was used to check the normality of distribution of the obtained values of data samples. Data with a normal distribution of values was represented by arithmetic mean (M) and standard deviation (SD). Significance of differences between the compared values in the case of normal distribution was determined by Student's t-test. In the absence of normal distribution, nonparametric criteria were used: Wilcoxon for pairwise comparisons of dependent variables, Mann–Whitney U test, and Pearson's chi-square test for comparisons of independent variables. Quantitative data with non-normal distributions was expressed as median (Me) and interquartile range (IQR, the interval between the 25th and 75th percentiles). Frequency and percentage (%) analysis was used to evaluate qualitative data. A p value <0.05 was taken as the threshold criterion for statistical significance between compared indicators. The effectiveness of primary prophylaxis of variceal bleeding (percentage of non-bleeding patients) and survival of patients in the compared groups (with and without NSBBs) were determined by Kaplan–Meier estimate with calculation of logarithmic Log-Rank (Mantel–Cox) test that determines differences between the corresponding curves.

RESULTS

Table 1 and Table 2 present data on demographic, clinical, laboratory parameters, and indicators (MELD-Na, CTP) in the groups of patients with ascites who received NSBBs (n = 97) and who did not receive NSBBs (n = 91) while waiting for LTx.

Table 1

Comparative characteristics of indicators of NSBB and non-NSBB patients (normal and non-normal distribution)

Indicator	NSBB (n = 97) M ± SD	No EVL (n = 91) M ± SD	Statistical significance
Normal distribution (M ± SD)			
Age	49.78 ± 12.19	46.41 ± 12.89	NS
Hemoglobin (g/L)	117.45 ± 24.11	114.59 ± 24.87	NS
White blood cells ($\times 10^9$/L)	3.21 ± 0.81	3.24 ± 0.75	NS
Platelets ($\times 10^9$/L)	78.57 ± 34.91	72.45 ± 36.89	NS
Serum albumin (g/L)	35.19 ± 4.84	32.81 ± 4.92	NS
MELD-Na	22.01 ± 4.35	20.35 ± 5.67	NS
Non-normal distribution (Me; IQR)			
INR	2.01 (1.57–2.52)	1.99 (1.64–2.47)	NS
Bilirubin (μmol/L)	71.5 (58.00–92.1)	68.1 (52.24–89.03)	NS
Creatinine (μmol/L)	91.2 (64.51–123.1)	89.6 (60.8–122.5)	NS
Na (mmol/L)	132.7 (117.1–154.0)	137.7 (103.9–176.1)	NS

Note: NS, no statistically significant difference ($p > 0.05$) between the compared parameters.

As can be seen from the data presented in these tables, the compared patient groups did not differ in terms of demographics, clinical, laboratory parameters, or structure of etiology of cirrhosis. There were no significant differences when comparing MELD-Na scores and the incidence of grade B and C in determining liver severity by CTP. Patients in both groups did not differ in terms of incidence of grade 2 and grade 3 ascites. Comparable groups had no statistically significant differences in the incidence of medium-sized (second degree) and large-sized (third degree) esophageal varices.

Thus, prior to therapy, the compared groups were comparable in terms of demographic, clinical, and laboratory parameters, etiology of cirrhosis, severity of liver lesions, severity of ascites, and incidence of medium- and large-sized esophageal nodules.

While waiting for liver transplantation for 1.5 months to 36 months, 138 patients in the compared groups developed variceal bleeding. In the group of patients treated with NSBBs during this period, bleeding esophageal varices developed in 51 patients, while in the non-NSBB group, bleeding developed in 87 patients (52.6% and 95.6%, respectively, p = 0.0001). These differences when comparing the NSBB and non-NSBB groups are demonstrated by a comparative analysis of the percentage of non-bleeding patients obtained using the Kaplan–Meier estimate with the definition of the log-rank test (p = 0.0001) (Fig. 1).

Table 2

Comparative characteristics of parameters of NSBB and non-NSBB patients

Indicator	NSBB (n = 97) (%)	No NSBB (n = 91) (%)	Statistical significance
Male	57 (58.8%)	52 (57.1%)	NS
Virus-induced cirrhosis	57 (58.8%)	53 (58.2%)	NS
Alcohol-induced cirrhosis	40 (41.2%)	38 (41.8%)	NS
Ascites, grade 2	67 (69.1%)	65 (71.4%)	NS
Ascites, grade 3	30 (30.9%)	26 (28.6%)	NS
Varicose veins, grade 2	62 (68.1%)	63 (69.2%)	NS
Varicose veins, grade 3	35 (31.9%)	28 (30.8%)	NS
CTP, class B	7 (7.2%)	8 (8.8%)	NS
CTP, class C	90 (92.8%)	83 (91.2%)	NS

Note: NS, no statistically significant difference (p > 0.05) between the compared parameters.

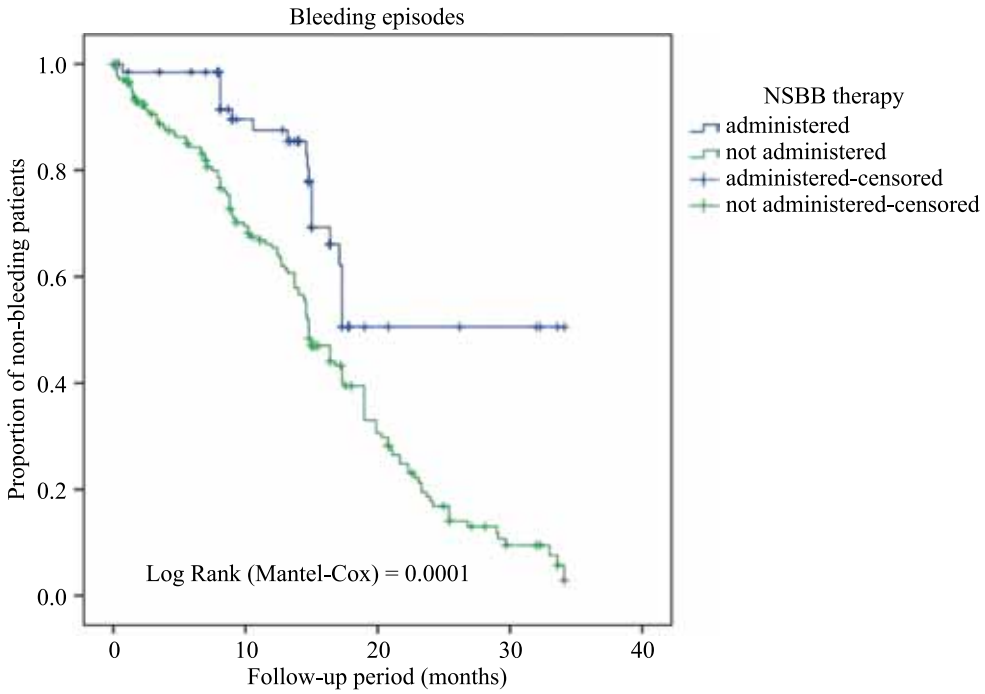

Fig. 1. Proportion of non-bleeding patients with and without NSBB therapy (Kaplan–Meier estimate with Log-Rank test). NSBB, non-selective beta-blockers; Log-Rank (Mantel–Cox) test, log rank nonparametric test for comparing survival curves, p = 0.0001

During this LTx waiting period, 145 patients died (58 from the NSBB and 87 from the non-NSBB group). Patient survival (Fig. 2) was significantly higher in the NSBB group than in the non-NSBB group (40.2% and 4.4%, respectively, p = 0.0001).

DISCUSSION

The progressive course of cirrhosis, characterized by a transition from a compensated to a decompensated form, manifested by the development of ascites, varicose vein bleeding and gastric variceal bleeding, is associated with an inordinately high increase in mortality [14]. In our study, we met patient selection criteria for primary prophylaxis of bleeding in patients with decompensated cirrhosis, ascites and non-bleeding varicose veins. In particular, the consensus on treatment of CSPH and its complications recommends primary prevention of bleeding in order to reduce the likelihood of further cirrhosis decompensation and mortality in patients with ascites and medium- and large-sized varicose veins [1, 11].

Unfortunately, in some patients, this therapy was not possible due to contraindications or intolerance of the drugs. It is known that some patients with cirrhosis have absolute or relative contraindications to traditional NSBBs, in particular those with peripheral vascular diseases, diabetes mellitus, chronic obstructive pulmonary disease, and bronchial asthma [15]. In this case, EVL is recommended in patients with ascites and CSPH to prevent bleeding and further decompensation of cirrhosis [1, 11]. In our study, a part of patients refused to use this intervention, which was the reason for them being included in the comparison group.

In our study, bleeding incidence was significantly lower in patients with ascites who received NSBBs as primary prophylaxis of bleeding than in the group of patients who did not receive this intervention. It should be emphasized that patients with ascites represent a group of patients at very high risk of variceal bleeding, and other life-threatening complications of cirrhosis, as they have a high hepatic venous pressure gradient (HVPG) [16].

It has been found that NSBBs are not effective in all patients with ascites. For example, of 452 patients with ascites, only 188 cirrhotic patients (42%) responded to NSBBs (a >20% decrease from baseline), resulting in lower odds of bleeding varicose veins, refractory ascites, spontaneous bacterial peritonitis, or hepatorenal syndrome [17]. In another study, Paternostro et al. [18] reported that HVPG-response to NSBBs within 90 days was achieved in 55.3% with cirrhosis and varices. The authors emphasized that absolutely all bleeding events occurred in HVPG-NSBB non-responders.

How can one identify among patients who will potentially respond to NSBB therapy and those who will not? The gold standard for monitoring hemodynamic response to NSBB therapy and investigating the severity of portal hypertension is the invasive method for determining HVPG [11, 19]. Decrease in HVPG level below 12 mm Hg or by 10% during primary prophylaxis of bleeding indicates **chronic** hemodynamic response to oral NSBBs [20]. However, determining this response requires repeated (second) invasive measurement of

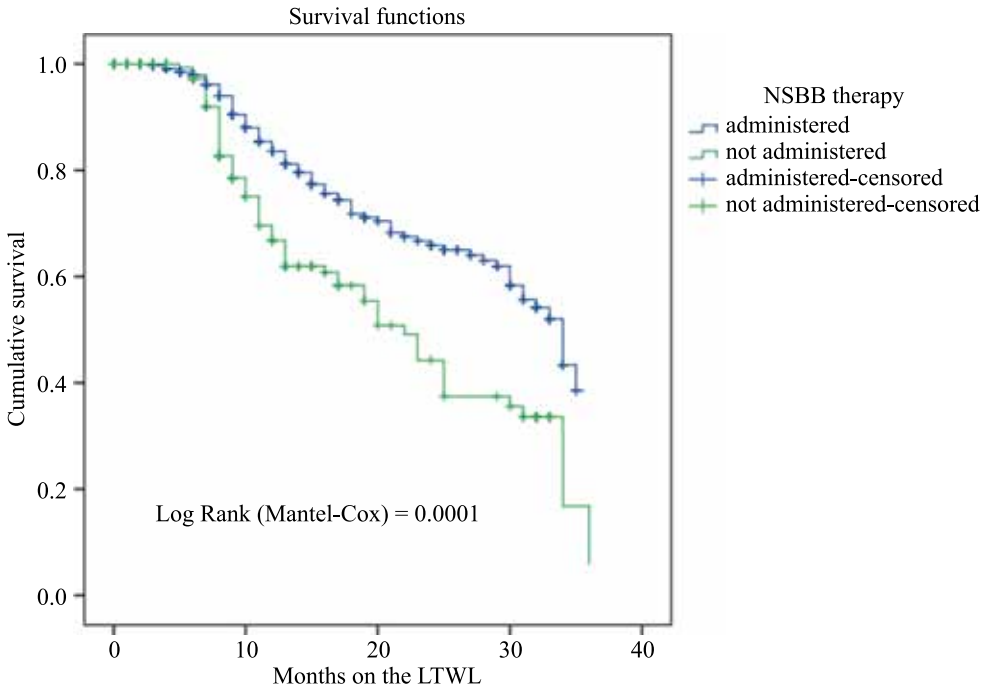

Fig. 2. Survival of patients in the NSBB and non-NSBB groups (Kaplan–Meier estimate with Log-Rank test). NSBB, nonselective beta-blockers; Log-Rank (Mantel–Cox) test, log rank nonparametric test for comparing survival curves, p = 0.0001

HVPG, and in some cases, between the first and second measurements, patients develop varicose vein bleeding, making it difficult to assess the chronic hemodynamic response to NSBBs [21]. An alternative to the first method has been proposed – to study of **acute hemodynamic response** to intravenous propranolol (≥10% reduction in HVPG levels) that helps to eliminate the disadvantages of the first method, potentially predicting the development of **chronic hemodynamic response** to oral NSBBs [22, 23].

So, the study of acute hemodynamic response to intravenous propranolol allows to stratify the risk of bleeding varicose veins at the early stage of portal hypertension during a single invasive study, reducing the need for repeated HVPG measurements [22, 23]. It has been found that acute hemodynamic response to propranolol during primary prevention of varicose vein bleeding actually reduces not only the development of the first bleeding, but also reduces the progression of ascites to more severe forms, development of ascites refractory to diuretics, reduces the development of spontaneous bacterial peritonitis and hepatorenal syndrome [20, 22].

Hofer et al. [24] found that acute hemodynamic effect on intravenous propranolol in patients with cirrhosis and CSPH is associated with a significant reduction in the risk of bleeding and hepatic decompensation. The authors confirmed different categories of patients when evaluating their response to NSBB therapy. In patients with decompensated cirrhosis, acute hemodynamic response on propranolol (58.2% of patients) were associated with a reduced risk of variceal bleeding at 12 months follow-up (3.6% responder; 15% nonresponder, log-rank, $p = 0.038$).

The disadvantages of our study include the limited technical capabilities of the center in determining HVPG, and, consequently, the absence of the possibility of acute or chronic hemodynamic response to NSBBs. In this regard, we can assume that significant development of bleeding in the NSBB group in our study is related to the presence of a category of HVPG-NSBB non-responders.

We found that the survival rate of patients with ascites who received NSBBs while waiting for LTx was significantly higher than the survival rate of cirrhotic patients whose therapy did not include NSBBs.

The effect of NSBBs on the survival of patients with decompensated cirrhosis is mixed. Conclusions by authors who have studied this problem vary, and, at times, contradict directly. For example, Sersté et al. [25] found that the 1-year survival of patients with refractory ascites decreased in the group of patients receiving propranolol. Kalambokis et al. [26] conducted a retrospective study of patients with cirrhosis of mixed etiology (96 CTP B and 75 CTP C), including alcoholic, viral and other etiologies, who had not previously received NSBBs. There were no significant differences in both groups (NSBB and non-NSBB) when comparing by gender, etiology of cirrhosis, and MELD score. Compared with those who did not receive NSBBs therapy, there was a significantly higher increase in 2-year mortality in the group with CTP B receiving this therapy. In the short-term follow-up (up to 6 months), there was a significant increase in mortality in the NSBB group than in the non-NSBB group among patients with CTP C or a MELD score of 16. Calès et al. [27] investigated the effect of NSBBs on liver-related mortality in a study of patients with alcohol-induced cirrhosis for over 5 years of follow-up. The authors found that the use of NSBBs (propranolol) reduced survival in patients with alcohol-induced cirrhosis associated with liver disease (MELD ≥12) compared with patients who did not receive these drugs. At the same time, NSBBs increased non-liver survival compared with patients without NSBB therapy.

A number of scientific publications have failed to note the effect of NSBBs on patient survival [28–30]. For example, Snoga et al. [30] stratified patients into those receiving and not receiving NSBBs therapy with the study of 24-month mortality in both groups of patients. The NSBB group and the non-NSBB group had similar patient mortality at 24 months (32.0% and 38.5%, respectively, $p = 0.51$). There were no significant differences in the proportion of bleeding and the proportion of patients who died from CSPH progression. In multivariate logistic regression, NSBB therapy was not a predictor of 24-month mortality.

A significant number of studies have found improved patient survival when comparing groups of cirrhotic patients receiving and not receiving NSBB therapy [31–33]. For instance, Ngwa et al. [31] investigated NSBB impact on the survival of patients enrolled in LTWL. NSBB use was associated with lower 90-day mortality (6% vs. 15%, $p = 0.03$). Patients taking NSBBs developed acute kidney injury (AKI) within 90 days more frequently (double) than patients not taking NSBB (22% vs 11%, $p = 0.048$). Twenty-seven percent of patients with >90 day follow up discontinued NSBB, most commonly for hypotension and AKI, had increased subsequent MELD and mortality. Sharma et al. [32] showed that survival of patients with large-sized varicose veins with primary prophylaxis of bleeding using NSBBs increased.

CONCLUSION

NSBBs constitute an efficacious therapy in the primary prophylaxis of variceal bleeding, thereby saving life and preventing attrition of patients with ascites from the liver transplant waitlist. The introduction of patient selection techniques for NSBBs by means of acute hemodynamic response testing on propranolol when measuring HVPG is a promising measure that significantly improves prognostic response in primary prophylaxis of bleeding.

The authors declare no conflict of interest.

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EXPERIENCE WITH PERCUTANEOUS RIGHT VENTRICULAR SUPPORT IN THE EARLY POST-LEFT VENTRICULAR ASSIST DEVICE IMPLANTATION PERIOD (CLINICAL CASE REPORT AND LITERATURE REVIEWS)

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Implantable left ventricular assist device (LVAD) is a state-of-the-art treatment for adults and children with end-stage heart failure. The early and late period after LVAD implantation can be severely complicated. Right ventricular failure (RVF) still remains a common complication after LVAD implantation. RVF is the cause of reduced post-implant survival. We suggest that an additional temporary or permanent right ventricular assist device (RVAD) is an effective treatment for LVAD-associated RVF. In this clinical case report, we describe the medical history of a pediatric patient (14 years old) with severe heart failure (PediMACS Level 1) against a background of dilated cardiomyopathy. The patient required peripheral venoarterial extracorporeal membrane oxygenation (VA-ECMO) prior to urgent LVAD (HM3) implantation. In the early post-LVAD implantation (1 POD) period, the patient presented with hemodynamic and echocardiographic events of acute RVF that was resistant to drug therapy (inotropic/vasopressor support, iNO) and required mechanical circulatory support (MCS) with a preoperatively implanted VA-ECMO. In the LVAD-associated RVF scenario, VA-ECMO as a means of total cardiac bypass is a non-physiological MCS method and, therefore, undesirable. In this clinical situation, our solution was to use a paracorporeal centrifugal blood pump for temporary right heart support. A RVAD was assembled using percutaneous cannulation in two sites and a modification of the pre-existing VA-ECMO circuit. For RVAD, we used an ECMO cannula previously installed through the femoral vein (26 F) and added a reverse venous cannula (22 F) through the right internal jugular vein into the pulmonary trunk. To facilitate the passage of the return cannula into the pulmonary artery, we used a contralateral sheath (6 F, 40 cm) and an Amplatz Super Stiff guidewire under radiological control. The oxygenator was removed from the circuit on day 2 of RVAD. Central hemodynamics (reduction in right atrial pressure (RAP) to 10 mm Hg, increase in pulmonary capillary wedge pressure (PCWP) to 14 mm Hg), as well as right ventricular (RV) and left ventricular (LV) volume characteristics all improved. These observations allowed us to optimize the performance of the implantable LVAD (increase in flow rate to 4.2 l/min or 2.1 l/min/m^2). The duration of paracorporeal RVAD after LVAD implantation was 7 days with an average flow rate of 2.3 ± 0.2 l/min. Postoperative treatment in the intensive care unit (ICU) lasted for 15 days. The patient was discharged from the hospital on postoperative day 34.

Keywords: right ventricular assist device, left ventricular assist device, heart failure, VA-ECMO.

INTRODUCTION

Implantable left ventricular assist device (LVAD) has become widely used in the last decade not only for long-term mechanical circulatory support (MSC) before heart transplantation (HT), but also as an independent method of definitive (final) treatment of chronic heart failure (CHF) that is poorly amenable to medication [1]. There is considerable experience of successful long-term application of implantable LVADs both in adults and in children of different age categories in order to survive till HT, restore their own heart or for lifelong MSC [2]. The 2-year patient survival after LVAD implantation has reached and is no longer statistically different from that of heart transplant recipients. With this, the use of implantable LVADs can be considered as an alternative method of CHF treatment [3].

However, despite the progress and achievement of high efficiency, long-term MCS implemented through implantable LVAD is associated with the risk of both early and late post-implant complications, which can negatively affect patient survival. One of such serious complications is RVF, which can occur in the early post-LVAD implant period and require urgent treatment measures, including application of short-term MCS by various right ventricular support techniques [4]. Chronic right ventricular dysfunction (poorly amenable to medical correction and accompanied by long-term dysfunction of implantable LVAD) in patients with previously

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implanted LVAD is an indication for implantation of a second pump in order to implement RVAD and improve central and systemic hemodynamics [5].

The aim of this report is to present our own experience with percutaneous right ventricular support for correction of RVF developing in the early post-LVAD implantation period.

MATERIALS AND METHODS

Between September 2021 and November 2022, LVAD was implanted in 16 pediatric patients (<18 years old). Two (12.5%) of 16 patients developed severe RVF in the early-post LVAD implantation period, requiring additional MCS by paracorporeal RVAD. In one case, RVAD was connected by percutaneous cannulation – an example and discussion are presented in this report.

CLINICAL CASE

Patient A.A.V., 14 years old (height 180 cm, weight 80 kg, body surface area 2.0 m^2, body mass index 24.7 kg/m^2) was admitted at Shumakov National Medical Research Center of Transplantology and Artificial Organs with the following diagnosis: dilated cardiomyopathy, relative tricuspid and mitral regurgitation. Thromboembolism of lower lobe branch of pulmonary artery of unknown age, multiple organ failure syndrome, CHF class IIB according to the Strazhesko–Vasilenko classification, NYHA functional class 4.

At the time of admission, the patient's condition was considered critical, which was due to severe acute decompensation of CHF with the development of multiple organ dysfunction syndrome (MODS), the leading components of which, apart from impaired heart pumping function, was clinically significant hepatorenal syndrome. Given the instability of systemic hemodynamics, the need for cardiotonic therapy (dopamine hydrochloride 8 μg/kg/min, epinephrine hydrochloride (adrenaline) 40 ng/kg/min), progression of MODS and metabolic disorders, short-term MCS by peripheral venoarterial extracorporeal membrane oxygenation (VA-ECMO) was used. Data from invasive central hemodynamic study, transthoracic echocardiographic and laboratory examination of the patient before the use of peripheral VA-ECMO are presented in Table 1.

Table 1

Results of invasive study of central hemodynamics, laboratory and instrumental examination (clinical case report, patient A.A.V., 14 years old)

Parameter	Investigation stages					
	Before VA-ECMO	Before iLVAD	After LVAD implantation and before RVAD	Against the background of both LVAD and RVAD	After RVAD removal	Before discharge
1	2	3	4	5	6	7
Examination of central hemodynamics						
HR (mins)	118	124	131	124	112	108
SBP / mAP / DBP (mmHg)	79 / 69 / 55	85 / 72 / 63	74 / 71 / 68	84 / 79 / 76	89 / 82 / 76	79 / 75 / 69
RAP (mmHg)	18	10	19	9	12	11
PASP / mPAP / PADP (mmHg)	36 / 33 / 26	34 / 29 / 22	35 / 30 / 26	35 / 26 / 16	37 / 24 / 14	–
PCWP (mmHg)	18	14	6	12	14	–
CO (l/min)	2.2	2.9	2.4	4.4	4.1	–
CI (l/min/m^2)	1.1	1.5	1.4	2.2	2.05	–
Δ PADP – PCWP (mmHg)	8	8	20	4	10	–
SV (ml)	17.8	23.4	18.3	35.5	36.6	–
SVi (ml/m^2)	8.9	11.7	9.2	17.8	18.3	–
TPG (mmHg)	15	15	24	14	10	–
RAP / PCWP	1.00	0.71	3.17	0.75	0.9	–
PVR (Wood units)	6.8	5.17	10.0	3.18	2.17	–
PVR (dynes/sec/cm^5)	545	414	800	254	174	–
RVSWI (gm/beat/m^2)	1.8	3.0	1.4	4.1	3.0	–
Dopamine (μg/kg/min) Dobutamine (μg/kg/min) Adrenaline (ng/kg/min)	8 – 40	6 – 10	6 4 20	6 2 20	6	– – –
VA-ECMO, Blood flow (l/min) Number of pump revolutions (rpm)	 – –	 3.3 6000	 2.4 4900	 – –	 – –	– – –
iLVAD Pump Flow (l/min) Pump Speed (rpm) Pulse Index Pump Power (watt)	 – – – –	 – – – –	 1.8 4650 2.2 2.9	 3.8 4600 4.4 3.2	 3.8 4950 5.0 3.3	 3.6 4800 5.3 2.6

End of Table 1

1	2	3	4	5	6	7
Transthoracic/transesophageal echocardiography						
Ao (annulus fibrosus) (cm)	1.9	1.9	1.9	1.9	1.9	1.9
Ao (ascending aorta) (cm)	2.6	2.6	2.6	2.6	2.6	2.6
LA (cm)	4.0	3.9	2.8	3.2	3.3	3.2
LA (ml)	80	74	38	53	56	51
RA (ml)	80	56	92	53	62	64
RV (cm)	3.4	2.9	3.6	3.1	3.2	3.3
RVF1	3.2	2.6	3.4	2.7	3.6	3.7
RVF2	3.0	2.9	3.2	2.5	3.2	3.2
RVF3	6.2	4.4	6.0	4.9	5.8	6.2
IVS (cm)	0.8	0.8	0.8	0.8	0.8	0.8
PW (cm)	0.8	0.8	0.8	0.8	0.8	0.8
LVEDV (ml)	200	182	71	88	84	98
LVESV (ml)	176	151	58	68	66	76
SV (ml)	24	31	13	20	18	22
LVEF (%)	12	17	18	22	21	22
Mitral valve regurgitation (grade)	2.5	3.0	2.5	2.5	2	2
Tricuspid regurgitation (grade)	2.0	2	3.0	2.0	2	2
TAPSE (mm)	18	17	8	11	14	14
Laboratory investigation						
Leukocytes,	13.9	10.7	16.2	17.8	14.5	9.3
stab cells (%)	1.0	3	7	5	1	2.0
segmented cells (%)	82.0	87	81	77	87	68.7
eosinophils (%)	0.5	0.1	0.0	1.4	2	1.5
basophils (%)	0.2	0.1	0.1	0.6	0	0.8
lymphocytes (%)	9.0	8	7	7.3	4	21.0
monocytes (%)	8.0	2	5	8.5	6	6.0
Hemoglobin (g/dL)	10.0	9.0	7.9	8.9	9.0	8.1
Red blood cells (10^{12}/L)	3.5	3.14	2.79	2.94	2.97	2.6
Hematocrit (%)	31.0	27.4	24.6	27.4	27.9	26.0
Platelets (10^9/L)	72	75	48	41	74	92
Albumin (g/L)	32.3	44.5	42.5	35.6	36.5	35.1
Total protein (g/L)	67.3	71.4	72.3	67.7	66.3	71.3
Total bilirubin (μmol/L)	40	32.6	32.8	38.5	36.1	10.9
ALT (U/L)	255	171.3	73.8	27.0	24.8	19.6
AST (U/L)	152	35.2	89.5	32.3	30.4	39.2
Urea, mmol/L	22	21.2	20.1	12.2	15.0	5.6
Creatinine (μmol/L)	112	110.8	75.1	78.0	89.1	31.7
PI (%)	64	55	66	65	53	40
INR	1.7	2.05	1.65	1.68	2.15	3.0
Fibrinogen (g/dL)	2.25	1.72	1.50	1.62	1.35	3.93
$pH_в$	7.274	7.541	7.437	7.499	7.439	7.382
$BE_в$ (mmol/L)	–5.8	10.2	1.9	2.9	3.9	2.2
$P_вCO_2$ (mmHg)	47.9	48.6	38.8	43.6	41.9	37.9
$P_вO_2$ (mmHg)	27.1	26.4	26.7	27.6	32.6	33.4
$S_вO_2$ (%)	48.6	40.4	46.5	49.6	58.7	62.3
Lactate (mmol/L)	3.9	2.1	5.8	1.5	1.6	1.2
Glucose (mmol/L)	5.7	7.1	7.2	6.5	6.7	5.3
K^+ (mmol/L)	3.8	4.0	4.6	4.2	3.9	4.3
Na^+ (mmol/L)	131	144	138	143	135	138

Note: HR, heart rate; SBP, systolic blood pressure; mAP, mean arterial pressure; DBP, diastolic blood pressure; RAP, right atrial pressure; PASP, pulmonary artery systolic pressure; mPAP, mean pulmonary artery pressure; PADP, pulmonary artery diastolic pressure; PCWP, pulmonary capillary wedge pressure; CO, cardiac output; CI, cardiac index; SV, stroke volume; SVi, stroke volume index; TPG, transpulmonary pressure gradient; PVR, pulmonary vascular resistance; RVSWI, right ventricular stroke work index; VA-ECMO, venoarterial extracorporeal membrane oxygenation; iLVAD, implantable left ventricular assist device; Pump Flow, iLVAD flow rate; Pump Speed, pump rotational speed; Ao, aorta; LA, left atrium; RA, right atrium; RV, right ventricle; RVF1, RVF2, RVF3, end-diastolic size of the RV at the basal and midline sections, and longitudinal size of the RV; IVS, interventricular septum; PW, left ventricular posterior wall; LVEDV, left ventricular end-diastolic volume; LVESV, left ventricular end-systolic volume; LVEF, left ventricular ejection fraction; TAPSE, tricuspid annular plane systolic excursion; ALT, alanine aminotransferase; AST, aspartate aminotransferase; PI, prothrombin index; INR, international normalized ratio.

The patient was connected to ECMO circuit via percutaneous puncture cannulas inserted into the right femoral vein (venous cannula 26 F) and the right femoral artery (arterial cannula 15 F). To prevent right lower extremity ischemia, the right superficial femoral artery was catheterized using a 15 G single-lumen catheter connected through a perfusion line (trunk) to the arterial part of the VA-ECMO circuit. VA-ECMO parameters were as follows: centrifugal pump speed, 6600 rpm; volumetric blood flow rate, 3.3 L/min or 1.6 L/min/m^2; flow gas (sweep gaze) volume rate, 3.5 L/min; flow gas FiO_2, 0.80.

Given the increased risk of heart transplantation (HT) against the background of high pre-transplant pulmonary hypertension (PH) (TPG, 15 mmHg; PVR, 6.8 and multiple organ dysfunction), LVAD was implanted as a method of long-term pre-transplant MCS and regression of PH. From the moment of admission at Shumakov Center until the time of surgery, the patient was on renal replacement therapy by continuous veno-venous hemofiltration, which was then continued in the intra- and postoperative period. The patient was assigned a Pedimacs I preimplantation level. Short-term MCS by peripheral VA-ECMO before LVAD implantation lasted for 5 days. Against the background of VA-ECMO application, metabolic and multiple organ disorders regressed. LVAD was implanted from the median sternotomy approach under cardiopulmonary bypass (CPB) and on a beating heart. Before the operation, the venous cannula of the VA-ECMO circuit was lowered caudally to 35 cm at the level of percutaneous inlet to prevent competition with the CPB venous cannula inserted into the inferior vena cava. During CPB, VA-ECMO flow rate was 0.8–1.0 L/min with a proportional decrease in the volume rate of CPB and the volume rate of flow gas supply to the membrane oxygenator of the CPB circuit. CPB lasted for 67 minutes. An LVAD HeartMate III model by Abbott Corp. (USA) with a centrifugal pump functioning on the principle of magnetic levitation was implanted. Inhaled nitric oxide (iNO) at 20 ppm dose was used as a selective pulmonary vasodilator to reduce increased pulmonary vascular tone and prevent the development of right ventricular failure (RVF) in the early post-implantation period.

At the end of the surgical stage of LVAD implantation, we started a stepwise (by 1 l/min) decrease in the CPB volume rate at a constant VA-ECMO volume rate (0.8–1.0 l/min) against gradual increase in the rate of revolution of the implanted LVAD (by 200 rpm) under control of its flow rate and echocardiographic parameters of filling and contractility of the right and left ventricles of the heart and the interventricular septum location. In the early post-implantation period, despite significant cardiotonic (dopamine hydrochloride 6.0 μg/kg/min, dobutamine hydrochloride 4.0 μg/kg/min, epinephrine hydrochloride 60 ng/kg/min) and vasodilator

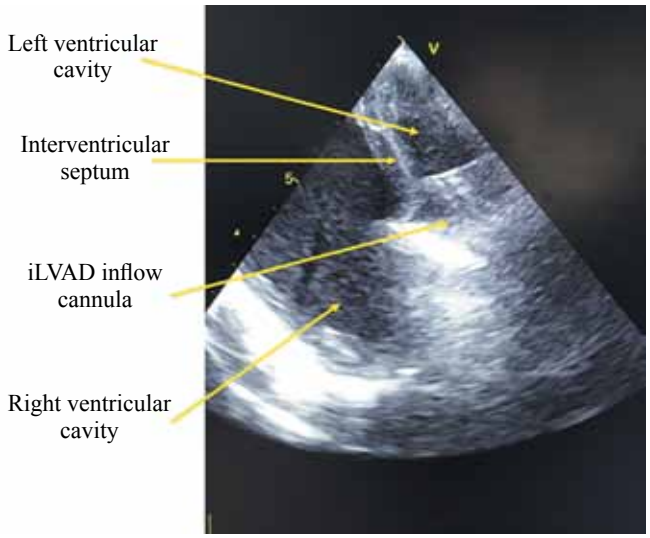

Fig. 1. iLVAD flow cannula suction to the interventricular septum against the background of acute RVF, accompanied by an increase in its volume with a simultaneous reduction in the left ventricular cavity (transesophageal echocardiography). iLVAD, implantable left ventricular assist device

(iNO) therapy at a constant VA-ECMO volume rate (0.8–1.0 L/min), acute right ventricular therapy was noted, which was manifested by increase in RAP to 19 mm Hg, decrease in PCWP to 6 mmHg with a simultaneous decrease in CI to 1.4 L/min/m^2 and LVAD flow rate to 2.2 L/min or 1.1 L/min/m^2. Transesophageal echocardiogram revealed increased RV volumetric characteristics, severe hypokinesis of its free wall and outlet section, up to grade 3 tricuspid regurgitation with simultaneous displacement of the interventricular septum toward the left ventricle, sharp reduction in left atrial and left ventricular cavity with LVAD suction cannula to the interventricular septum) (Fig. 1). In order to improve systemic blood flow against the background of acute RVF, the peripheral VA-ECMO volume rate was increased to 3.0 l/min. Given "unphysiological" MCS by peripheral VA-ECMO under LVAD conditions, leading to volumetric underload of the left heart and impaired heart function, it was decided to switch from peripheral VA-ECMO to percutaneous RVAD. The goal was to provide increased blood flow to the left heart and increase LVAD flow rate.

Percutaneous RVAD implantation technique

Under local anesthesia, the puncture and catheterization of the right internal jugular vein was performed, with a J-shaped endovascular contralateral introducer (40 cm, 6 F) with an internal stent (Fig. 2), guided through the right atrium into the RV cavity. Manipulations were performed under fluoroscopic control. A super-stiff metal conduit was passed through the J-shaped introducer sheath into the right heart and then into the right pulmonary artery (Fig. 3). After removal of the

introducer and subsequent dilation of the skin canal with percutaneous dilators, a venous ECMO cannula (22 F) was passed through the right internal jugular vein, the right heart into the pulmonary artery trunk to below 1 cm from the left and right pulmonary veins (Fig. 4). This cannula was connected to the VA-ECMO circuit arterial trunk separated from the arterial cannula to perform percutaneous RVAD according to the inferior vena cava-pulmonary artery scheme (Fig. 5). After RVAD initiation, the arterial cannula was extracted from the lumen of the right femoral artery.

Early post-LVAD implantation period against the background of paracorporeal RVAD

Against the background of RVAD, there was an improvement in central hemodynamics (decrease in RAP to 10 mmHg, increase in PCWP to 14 mmHg) and RV and LV volumetric characteristics. This allowed to optimize LVAD functioning (increase in flow rate to 4.2 l/min or 2.1 l/min/m^2). Postoperative MV lasted for 28 hours, postoperative application of renal replacement therapy by continuous veno-venous hemofiltration (CVVH)

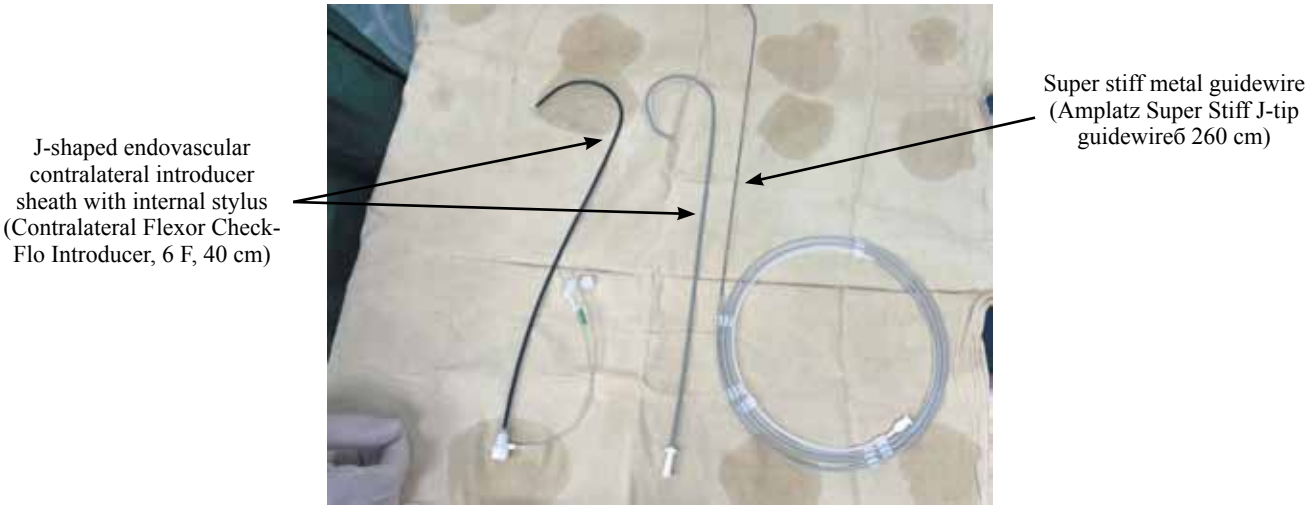

Fig. 2. J-shaped contralateral endovascular introducer sheath and super stiff endovascular guidewire for improved percutaneous venous cannulation from the right internal jugular vein into the pulmonary artery

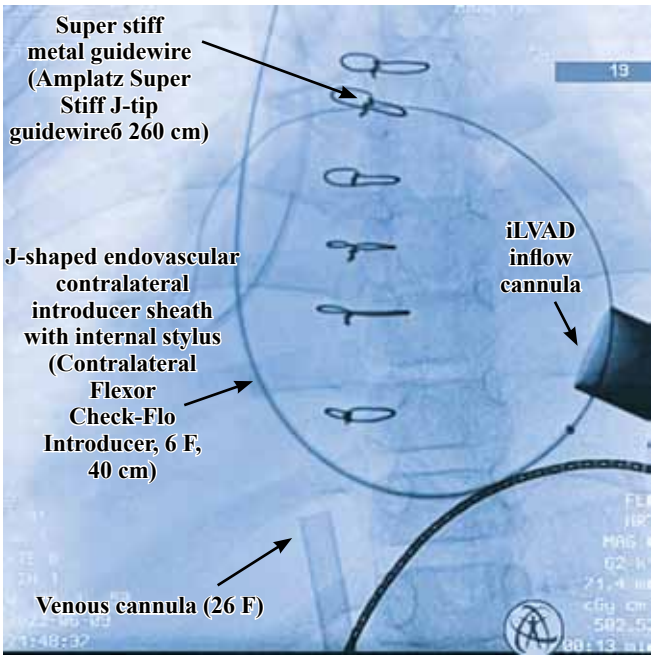

Fig. 3. Passage of a super stiff endovascular guidewire through the J-shaped endovascular contralateral introducer with an internal stylet into the pulmonary artery (fluoroscopic image)

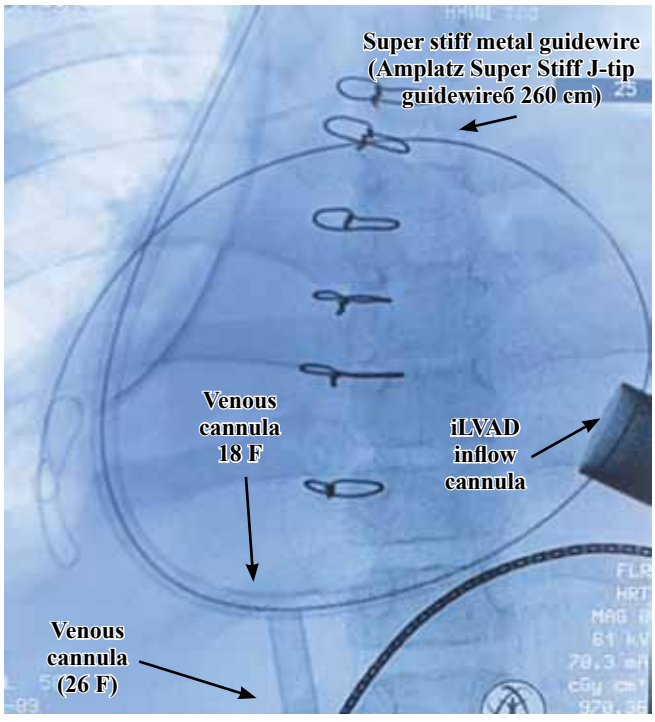

Fig. 4. Insertion of a return venous cannula via a super stiff endovascular guidewire through the right heart into the pulmonary artery (fluoroscopic image)

Acute right ventricular dysfunction that developed after LV assist device implantation and required paracorporeal right ventricular bypass under the "inferior vena cava – pulmonary artery" scheme

M., 14 years old

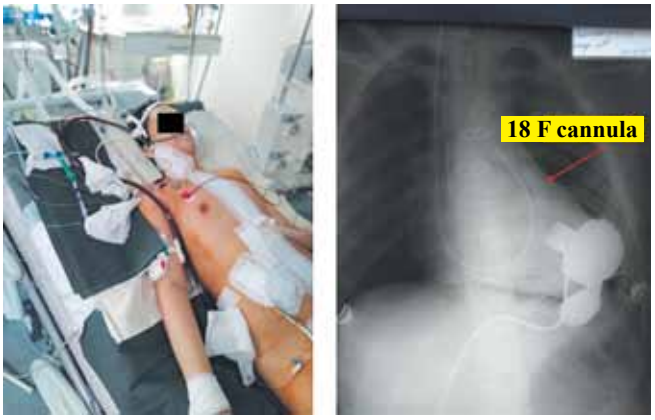

Fig. 5. The patient with percutaneous paracorporeal right ventricular assist device (left photo) and venous (outflow) cannula inserted through the right vena cava into the pulmonary artery (right, radiographic image)

lasted for 3 days. Membrane oxygenator remained integrated in the paracorporeal RVAD circuit for the first 2 days in order to control blood gas composition. The membrane oxygenator was then disconnected from the extracorporeal circuit in the absence of gas exchange function disorders in the lungs. Paracorporeal RVAD after LVAD implantation lasted for 7 days with an average flow rate of 2.3 ± 0.2 l/min. After gradual reduction in RVAD flow rate under the control of central hemodynamics and transthoracic echocardiography, cannulas were removed by purse-string suturing around the percutaneous inlet. After RVAD termination, there were no hemodynamic and echocardiographic signs of clinically severe RVF in the follow-up period (see Table 1). Postoperative treatment in the ICU took 15 days. The patient was discharged from the hospital on postoperative day 34. Baseline post-implantation drug therapy included: ivabradine 5 mg, acetylsalicylic acid 100 mg, warfarin 3.75 mg, sildenafil 25 mg twice daily. Implantable LVAD parameters at the time of discharge from hospital: pump flow 3.6 l/min or 1.8 l/min/m^2, pump speed 4800 rpm, pump power 2.6 watts, pulse index 5.3. At the time of writing, the patient was under outpatient care without clinical and echocardiographic manifestations of late RV dysfunction and with optimal functioning parameters of the implanted LVAD.

DISCUSSION

RVF is a frequent complication following LVAD implantation. It leads to impaired performance and, accordingly, to lower efficiency of MCS itself, multiple organ failure and death [6]. According to various studies, the incidence of acute RVF in the early period following LVAD implantation ranges from 17% to 50% [7, 8]. There is still no generally accepted definition of acute RVF resulting after LVAD implantation. Potapov E. et al. (2008) defined acute RVF as right ventricular pump function failure developing within the first 48 hours after LVAD implantation in the absence of hemopericardial tamponade. It is accompanied by the following manifestations: mean BP ≤55 mmHg, RAP ≥16 mmHg, mixed venous oxygen saturation (SvO_2) ≤55%, CI <2.0 L/min/m^2 despite inotropic support [9].

RVF is accompanied by decreased efficiency in the implantable LVAD due to decreased blood flow to the left heart and development of complications specific for this variant of MCS, such as hemolysis, aortic insufficiency, ventricular arrhythmias resulting from creation of increased negative pressure and inflow cannula suction to the left ventricular wall [10]. Besides, when the axial or centrifugal pump turns too frequently, excessive unloading of the left heart occurs, which, even in the absence of preexisting RVF, causes displacement of the interventricular septum, interventricular relationship disorder and its development, especially in hypovolemia [10].

Recently, depending on the period of occurrence after LVAD implantation, acute (<48 hours), early (>48 hours and <14 days) and late (>14 days) forms of RVF, differing in approaches to its drug and non-drug treatment, have been distinguished [11]. As the life expectancy of patients with implantable LVAD increases, the incidence of late RVF increases, with clinical and instrumental manifestations being detected in more than 40% of cases 2 years after implantation [12]. According to INTERMACs criteria, depending on RAP level, dosage and duration of inotropic and vasodilator therapy, RVAD requirement and risk of death, mild, moderate, severe and acute severe forms of RVF in patients with implantable LVAD are distinguished [13].

There are pre-implantation and peri-implantation risk factors of post-LVAD implantation RVF. Numerous parameters of preoperative laboratory, hemodynamic, echocardiographic and MRI examinations have been suggested to predict the development of RVF both early and late after LVAD implantation (Table 2) [7, 14–16]. High prognostic significance of underlying myocardial imaging (speckle-tracking echocardiography, magnetic resonance imaging) with determination of segmental and global RV deformation to predict its dysfunction in various clinical situations, including LVAD implantation, has been demonstrated [17].

Pre-existing RVF is the leading cause of exacerbation of RV pumping dysfunction after LVAD implantation [8]. Patients with preimplantation low TAPSE value, RV free wall strain, RVFAC and high values of RV/LV end-diastolic area ratios, left atrial (LA) volume index, LA diameter/LV end-diastolic diameter ratio (ratio have an increased risk of RVF after LV implantation [18]. Severe pre-implantation tricuspid regurgitation increases the risk of early RVF 3.5-fold in patients with LVAD [19]. The

relevant prognostic scales – Michigan RV failure risk score and Bayesian model – have been developed for predicting post-LVAD RVF [20, 21]. In patients with high risk of early or late severe RVF, biventricular implantable circulatory support should be considered as the method of choice for long-term MCS [22]. The outcome of long-term MCS in patients with delayed RVAD implantation is 4.8 times worse than the survival rates of patients with simultaneous LVAD and RVAD implantation [23]. It should be taken into account that RVF can also develop

Table 2

Preoperative clinical, laboratory and instrumental factors of RVF after LVAD implantation

Parameter groups	Factors
Clinical manifestations	Systemic circulatory insufficiency
	Ascites
	Liver dysfunction
	Kidney dysfunction
	Obesity
	Tachycardia
	Inotropic/vasopressor support
	Mechanical circulatory support
	Renal replacement therapy
	MV
Laboratory parameters	Total bilirubin ≥2.0 mg/dL
	AST ≥80 U/L
	Urea nitrogen >39 mg/dL
	Blood creatinine ≥2.3 mg/dL
	MELD
	Glomerular filtration rate by MDRD 4
Echocardiographic parameters	Right atrial size
	Anteroposterior RV end-diastolic area (D1, D2, D3)
	Indexed RV end-diastolic volume
	Indexed RV end-systolic volume
	RV ejection fraction
	Basal RV/LV end-diastolic area ratio (RVED1/LV) ≥0.75
	LA volume index
	LA diameter/LV end-diastolic diameter ratio
	RV fractional area change (RVFAC)
	Tricuspid regurgitation Grade 3.
	TAPSE <8 mm
	Tricuspid annular peak systolic velocity (TAPSV) <8 cm/sec
	Pulsed Doppler transmitral E wave (Em) / tissue Doppler lateral systolic velocity (SLAT) ≥18.5
	RV strain by speckle tracking technology: – RV global longitudinal strain (GLS) – RV free wall strain (RVFWS) >–11.8%…–9.6% – RV systolic longitudinal strain rate <0.6/sec
Hemodynamic parameters	CVP (RAP) >15 mmHg
	RAP/PCWP ratio >0.55
	Pulmonary arterial pulse pressure (PASP – PADP)
	Pulmonary artery pulsatility index: pulmonary arterial pulse pressure / RAP <2.0
	Pulmonary artery compliance: SV/pulmonary arterial pulse pressure
	Pulmonary effective arterial elastance (PASP/SV)
	Right ventricular stroke work index (RVSWI, right ventricular stroke work index) <0.25 mmHg/L × m^2
	Right ventricular systolic working index (RVSWI): pulmonary arterial pulse pressure – RAP/SVi
	Pulmonary vascular resistance (PVR)
	Simple right ventricular contraction pressure index (sRVCPI): TAPSE × (RV-RA pressure gradient) <400
MRI parameters	RV global longitudinal strain

Note: MV, Mechanical ventilation; AST, aspartate aminotransferase; MELD, Model for End-Stage Liver Disease; MDRD 4, Modification of Diet in Renal Disease; RV, right ventricular; LV, left ventricular; LA, left atrial; CVP, central venous pressure; RAP, right atrial pressure PASP, pulmonary artery systolic pressure; PADP, pulmonary artery diastolic pressure; PCWP, pulmonary capillary wedge pressure; SV, stroke volume; SVi, stroke volume index.

in patients without preimplantation risk factors due to perioperative RV myocardial damage of various genesis (embolism, mechanical trauma, etc.) [23].

The strategy for prevention and treatment of acute RVF following LVAD implantation includes tricuspid annuloplasty at the same time as implantation amidst severe insufficiency, strictly controlled volemic load, gradual increase in the implanted pump speed under strict echocardiographic control of the interventricular septum position, use of pulmonary vasodilators (inhaled nitric oxide, prostaglandin E1, prostacyclin, sildenafil) or inodilating effect (dobutamine, milrinone, levosimendan) in patients with high level of pulmonary vascular resistance, correction of hypoxemia and hypercapnia [24–26]. It should be taken into account that in patients without preimplantation pulmonary hypertension, the use of pulmonary vasodilator therapy is ineffective in acute RVF following LVAD implantation [27]. The combination of acute RVF with postperfusion vasoplegic syndrome requiring the use of high vasopressor support is associated with extremely high postoperative mortality [28].

Timely initiated MCS in ineffective drug treatment of acute RVF provides correction of systemic hemodynamic disorders, organ perfusion and prevention of the development of multiple organ disorders [4]. In conditions of implantable LVAD, the use of VA-ECMO should be considered a non-physiological method of MCS in the development of acute RVF, as it is an indirect bypass of the heart and leads to decreased blood flow to the left heart and malfunction of the implanted blood pump. In this scenario of acute RVF development from a hemodynamic point of view, it is more justified to use RVAD, which provides increased blood flow to the left heart, increased performance of the implanted LVAD and improved systemic circulation.

Currently, several extracorporeal RVAD techniques based on the use of external centrifugal pump of various modifications (Biopump Medronic, Rotoflow, CentriMag, Medos, TandemHerat-RV, etc.) or microaxial catheter pump (Impella-RP) are implemented in clinical practice. Accordingly, the central RVAD connection technique based on the centrifugal pump in the right atrium-pulmonary artery scheme, requiring repeated sternotomy during decannulation, or percutaneous cannulation technique using two single-lumen cannulas or one double-lumen cannula have been developed and are used in practice [29]. Percutaneous separate cannulation can be performed by passing an inflow and outflow cannula through the femoral veins (bifemoral approach) or inflow cannula through the femoral vein and outflow cannula through the right jugular vein. In both cases, the outflow cannula should be passed through the right heart and positioned in the lumen of the pulmonary artery [30]. Recently, the technique of percutaneous RVAD performed with a double-lumen cannula inserted through the right internal jugular vein and the right parts of the heart into the pulmonary artery (ProtekDuo) has been developed and introduced into clinical practice, to correct acute RVF developed in various clinical situations (heart transplantation, heart attack, acute post-cardiotomy heart attack, LVAD implantation, pulmonary embolism, high pulmonary hypertension, etc.) [31]. The double-lumen cannula facilitates RVAD through unilateral vascular access (internal jugular vein), which makes this MCS technique the least traumatic in acute RVF of postcardiac, postimplantation or posttransplantation genesis [32].

Timely initiation of MCS by RVAD provides better treatment outcomes for acute RVF following LVAD implantation compared to delayed start of assisted circulation [33]. In case of concomitant critical disorders in gas exchange function of the lungs (hypoxemia/hypercapnia), RVAD can be supplemented with ECMO. Separate studies demonstrate better survival rates when RVAD is combined with ECMO [34, 35]. It is necessary to maintain lower RVAD flow rate in relation to LVAD, whose level should not exceed 4.0 L/min in adult patients to ensure prevention of pulmonary edema or pulmonary hemorrhage [36]. The duration of RVAD use for acute RVF following LVAD implantation averages about 5 days [37].

Our own experience with LVAD implantation in pediatric patients (under 18 years of age) demonstrates that severe acute RVF, requiring the use of short-term MCS by paracorporeal centrifugal RVAD, developed in 12.5% of observations. This article provides an example of successful RVAD use in a 14-year-old patient. The possibility of percutaneous cannulation for RVAD (without the need for a re-sternotomy) to install and subsequently remove the inflow and outflow cannulas, carried out through the right femoral vein into the inferior vena cava and the right internal jugular vein into the pulmonary artery respectively, is demonstrated. Application of short-term RVAD improved the central hemodynamics, increased blood flow to the left heart and increased LVAD performance. RVAD duration for 7 days was sufficient to improve RV pumping function and discontinue its use of short-term MCS. In addition, this clinical observation demonstrates the continuity of assisted circulation in a patient with end-stage CHF and preimplantation INTERMACS Level 1 with consecutive transition from VA-ECMO to long-term MCS by implantable LVAD.

CONCLUSION

Short-term MCS by paracorporeal centrifugal RVAD is an effective remedy for hemodynamic disorders caused by early acute post-LVAD RVF. The percutaneous cannulation technique is a less traumatic way to perform right ventricular bypass.

The authors declare no conflict of interest.

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PEDIATRIC MECHANICAL CIRCULATORY SUPPORT: PATHOPHYSIOLOGY OF PEDIATRIC HEMOSTASIS AND POSTOPERATIVE MANAGEMENT ALGORITHMS

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Chronic heart failure (CHF) against the background of congenital heart disease, mostly in early childhood, or various forms of cardiomyopathies, more common in teenage age, represents an important cause of morbidity and mortality in the pediatric population [1, 2]. Due to the increase in the number of patients suffering from refractory end-stage CHF over the last two decades, and the current shortage of donor organs in pediatric practice, the issue of long-term mechanical circulatory support (MCS) is becoming increasingly a pressing problem. Patient management is a multidisciplinary task, since prolonged use of anticoagulant and antiplatelet therapy to prevent ventricular thrombosis has potentially life-threatening complications – acute hemorrhagic stroke and bleeding of varying severity.

Keywords: heart failure in children, long-term mechanical circulatory support, anticoagulant therapy, antiplatelet therapy.

GENERAL CHARACTERISTICS OF EXISTING SYSTEMS OF LONG-TERM MECHANICAL CIRCULATORY SUPPORT

Mechanical circulatory support (MCS) is a general term that currently describes various types of mechanical devices capable of fully or partially replacing the patient's heart function [3]. Specific definitions for different types of MCSs are applied depending on their purpose, duration of application, cannulation options and blood flow type. Long-term MCS systems include ventricular assist devices, which can replace the function of the left and/or right ventricles of the patient's own heart. The main characteristics of the currently available long-term MCS systems used in pediatric practice are presented in Table 1.

The first publications on successful implantation of continuous-flow long-term MCS systems in teenagers with end-stage CHF appeared in the early 2010s [4, 5]. In the initial stages of the program, these systems were implanted in children weighing 50 kg or more, taller than 150 cm, and with a body surface area (BSA) >1.5 m^2. Subsequently, the anthropometric criteria for implantation of such systems were expanded. For instance, according to the Fourth Annual Pediatric Interagency Registry for Mechanical Circulatory Support (Pedimacs), published in 2020, 1,031 MCS devices were implanted in patients under 19 years of age between September 19, 2012, and December 31, 2019. Intracorporeal continuous-flow long-term MCS devices were implanted in children aged 13.2 ± 3.9 years (n = 365). The number of paracorporeal continuous pumps implanted was 212, the average age of the patients was 3.6 years. In a group of children aged 2.7 years, pulsatile paracorporeal pumps were used most frequently (n = 230). It was shown that the incidence of cerebrovascular and hemorrhagic complications depends on the type of implanted device [6].

COMPLICATIONS ASSOCIATED WITH HEMOSTATIC DYSFUNCTION

Compared to the adult population, the pediatric cohort requiring the use of MCS devices is characterized by increased incidence of hemorrhagic and thrombotic complications, which is explained by changes in the hemostatic system, taking into account the age of the child, the specific course of some congenital heart defects, and the effect of components of the assisted circulation system on the coagulating blood system. Direct contact of the patient's blood with biomaterials of the system leads to activation of the complement system and production of inflammatory cytokines [7]. Normally, endothelial cells produce anticoagulant and procoagulant factors, and contribute to maintaining normal homeostasis in the body. Blood contact with non-endothelialized biomaterials activates a protective response, the degree of which may vary depending on the patient's individual characteristics. As a result, there is an imbalance of physiological coagulation due to increased consumption of

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anticoagulation factors and stimulation of procoagulant production [8, 9].

It should be noted that in the group of newborns and children in their first year of life requiring the use of MCS, the issue of anticoagulant therapy dosage selection is particularly relevant. This is primarily due to "developmental haemostasis" – physiological changes in the blood coagulation system, which depend on the child's age [10]. A specific feature of newborns is physiologically low level of vitamin K-dependent coagulation factors, such as factors XI and XII, prekallikrein, high-molecular-weight kininogen, whose level gradually increases and reaches the level of an adult by six months of life. Von Willebrand factor (vWF) and factor VIII levels have been shown to be elevated at birth and in the first 3 months of life [11, 12].

In addition to physiological age-related features in the hemostasis system, it is necessary to remember about acquired coagulation defects. For instance, young children with cyanotic congenital heart disease often have hypercoagulation syndrome on the background of polycythemia and increased levels of platelets, fibrinogen and coagulation factors V and VIII. In older people, such a condition leads to systemic circulation stasis against the background of CHF, which is accompanied by liver dysfunction with thrombocytopenia, decreased production of clotting factors and increased fibrinolytic activity [13].

The latest generation of long-term MCS devices contain biomaterials that allow maximum bio- and hemocompatibility when in contact with the human body. However, no surface of the systems used today has complete identity with the human endothelium.

A history of gastrointestinal angiodysplasia and congenital coagulopathy can alter the pharmacokinetics of antiplatelet and anticoagulant therapy in patients on long-term MCS.

Development of hemolysis against the background of direct exposure of erythrocytes to a mechanical heart pump, characterized by the presence of serum free hemoglobin >40 mg/dL and increased lactate dehydrogenase (LDH) levels, leads to production of vWF that plays an important role in impaired hemostasis against the background of ventricular heart operation [14, 15].

It has been shown that roller pumps, as well as short- and long-acting axial flow devices cause higher levels of hemolysis in comparison with centrifugal blood pumps. For example, according to the literature, higher baseline hemolysis levels were seen in patients after HeartMate II implantation compared with Heartware and HeartMate III [16]. Despite these findings, the choice of an implantable long-term MCS system, in pediatric practice, primarily depends on the child's anthropometric data [17].

ANTICOAGULANT THERAPY

To date, there are no unified guidelines for administration of anticoagulant and antiplatelet therapy in pediatric patients who are implanted with long-term MCS pumps. Standard management regimens for this category of patients include the use of unfractionated heparin (UFH) or low-molecular-weight heparin (LMWH) in the early postoperative period, followed by transition to warfarin in combination with antiplatelet agents (aspirin or clopidogrel).

HEPARIN USE IN THE EARLY POSTOPERATIVE PERIOD

The use of heparin in the early postoperative period is associated with the risk of complications of varying severity, especially in newborns and children in their first year of life, and, accordingly, a number of problems for the clinician.

1. Heparin dose variability makes it difficult to maintain a standard therapeutic dose of the drug [18].
2. Incidence of heparin-induced thrombocytopenia is about 1%, which is associated with an adverse prognosis for life [19, 20, 21].
3. Heparin is a biological compound, so allergic reactions of varying severity, up to and including anaphylactic shock with mortality, are possible [22].

Table 1

Long-term MCS systems in pediatric practice

Name	Pumping mechanism	Flow type	Maximum flow	Support type	Used in children
Syncardia TAH	Pneumatic	Pulsatile	7–9 L/min	Biventricular	Teenagers
Berlin Heart EXCOR®	Pneumatic	Pulsatile	3–7.2 L/min	LVAD, RVAD, Biventricular	>3 kg
Jarvik 2000 Jarvik 2015	Axial	Continuous	7 L/min 3 L/min	LVAD	Teenagers >30 kg >8 kg
HeartMate II	Axial	Continuous	3–10 L/min	LVAD	Teenagers
HeartMate III	Centrifugal	Continuous	10 L/min	LVAD, RVAD, Biventricular	Teenagers
HeartWare	Centrifugal	Continuous	10 L/min	LVAD	>15 kg

Note: LVAD, left ventricular assist device; RVAD, right ventricular assist device.

4. Prolonged use of heparin can lead to osteoporosis and spontaneous bone fracture due to inhibition of osteoblast formation [23].

To date, various monitoring tests have been proposed to evaluate the effectiveness of heparin therapy. The most common are presented in Table 2.

Ongoing heparin therapy in most cases is monitored by determining the aPTT.

It is known that a sufficient level of endogenous antithrombin III (AT III) is necessary for effective heparin therapy. However, in newborns, serum AT III levels are physiologically low, which may lead to ineffective heparinization and require administration of fresh frozen plasma or recombinant antithrombin drugs [24].

Bivalirudin, a direct thrombin inhibitor, inhibits all reactions catalyzed and induced by thrombin, including thrombin formation, activation of clotting factors V, VIII and XIII, activation of protein C and platelet aggregation. The effect of the drug is not related to the amount of circulating endogenous AT III in blood plasma, which allows for a more stable anticoagulant effect in this patient cohort [25].

Bivalirudin can be used:

– As an alternative to heparin in anticoagulant therapy, especially in heparin-induced thrombocytopenia (HIT);
– When it is impossible to achieve and maintain therapeutic aPTT values on the background of heparin infusion;
– In patients with signs of developing thrombosis despite heparin therapy [26].

When prescribing bivalirudin in the early postoperative period, the dose is selected depending on the risks of postoperative bleeding. At high risks of bleeding, it is recommended to maintain the target aPTT value at 50–60 s; at planned course of the postoperative period, the drug dose is titrated until the aPTT level reaches 60 to 80 s; at high risks of prosthetic heart valve thrombosis, maintaining the target aPTT value at 70 to 90 s is recommended [27].

To date, there have been few reports in the literature on administration of bivalirudin in patients after ventricular assist device implantation.

Today, there are three main protocols for anticoagulation therapy used in pediatric patients on long-term MCS (Table 3).

As can be seen from Table 3, each of the three protocols has its own differences, but they are all based on therapy that includes a combination of antiplatelet and anticoagulant drugs.

The Edmonton protocol was the first detailed guideline for anticoagulant and antiplatelet therapy in children after implantation of paracortical systems for long-term MCS and included administration of UFH, LMWH, warfarin, aspirin or clopidogrel. Heparin dose was adjusted according to the anti-Xa level and under control of aPTT, the target values of which were 1.5–2.5 times higher than the baseline values. Initiation of antiplatelet therapy was determined by thromboelastogram values [31].

In 2016, a prospective study by Steiner and coauthors analyzed the effectiveness of anticoagulant and antiplatelet therapy, the incidence of severe complications and the

Table 2

Tests for determining the effectiveness of heparin therapy

Test name	Method	Advantages	Disadvantages
Activated clotting time (ACT)	Whole blood clotting time is measured in seconds using activators (kaolin or celite)	Widely used in clinical practice Results are received within seconds Possibility of bedside testing	In some cases, ACT does not accurately reflect the effect of heparin due to an increase in the indicator regardless of the dose of the drug against the background of: – Hemodilution – Thrombocytopenia – Hypothermia – Hypofibrinogenemia – Lack Of Clotting Factors
Activated partial thromboplastin time (aPTT)	Internal and external pathways of the coagulation cascade are evaluated. It is carried out by adding an activator and phospholipid to the patient's plasma, followed by addition of calcium and measuring the clotting time in seconds	Widely used in clinical practice Rapid results	Decreased accuracy of results in vitamin K and/or factor VIII deficiency
Anti-factor Xa assay	A reagent and chromophore substrate are used to quantify the heparin-AT complex	Rapid results	Inaccurate assessment of the effectiveness of heparin in: – Hyperbilirubinemia – Hemolysis – Hyperlipidemia – High Antithrombin Levels

need for ventricular assist device replacement in children on paracortical long-term MCS who received therapy according to the Edmonton Protocol. Bleeding incidence was 43% of cases, of which in 24% of the cases, this complication was associated with the administered blood thinning therapy and was observed mainly during the first 14 days after surgical intervention. From day 4 to day 30 after implantation, 28% of patients were diagnosed with acute impairment of cerebral circulation and 9% of them were associated with ongoing drug therapy. The mechanical device had to be replaced in 56% of cases because of thrombotic complications. So, the authors concluded that new approaches for antiplatelet and anticoagulant therapy in children on long-term MCS are needed [32].

Ed Peng et al. analyzed the outcomes of implantations of Durable Continuous-Flow Mechanical Circulatory Support (HVAD; HeartWare, Framingham, MA) devices in 12 children between 2010 and 2015. The median age of the children was 7.1 years (3.7 to 17.0 years); four patients were aged 5 years or less. Indications for LVAD implantation in 11 cases were chronic heart failure that developed against a background of dilated cardiomyopathy, in one case the system was implanted due to cardiac graft dysfunction against the background of acute rejection. The mean length of support was 150 days (range, 16 to 638). Anticoagulant therapy was started 24 hours after implantation, provided the rate of drainage discharge was <1 ml/kg/hour for three consecutive hours. Revision of the mediastinum to stop bleeding was required in two patients. In two cases, device thrombosis was diagnosed without development of neurological complications. The 1-year actuarial survival was 100% with no neurologic events [33].

David N. Rosenthal et al. compared the effect of the Edmonton and Stanford protocols of anti-thrombotic therapy in children for extracorporeal long-term MCS on the incidence of acute cerebrovascular accidents. The Edmonton antithrombotic guideline cohort included children implanted before September 2012 when dual antiplatelet therapy was used with doses titrated to thromboelastography and/or platelet mapping. The second cohort of the Stanford Modified Antithrombotic Guideline cohort, which included children implanted on or after September 2012 when triple antiplatelet therapy was used routinely and where doses were uptitrated to high, weight-based dosing targets. So, target doses of aspirin, clopidogrel and dipyridamole were higher, with less dosing variability in the Stanford cohort than in the Edmonton cohort ($p < 0.003$). The incidence rate of stroke in the Stanford cohort was 84% lower than in the EG cohort (0.8 vs 4.9 events per 1,000 days of support, $p = 0.031$), and 86% lower than in the previous Investigational Device Exemption trial ($p = 0.006$) [34].

To date, the latest generation of the HeartMate III (Abbott Corporation) continuous-flow intracorporeal pump has been actively implanted in children with end-stage CHF. Matthew J. O'Connor et al. in their paper analyzed the results of HeartMate III implantations in 35 patients aged 8.8 to 47.3 years (median age of 15.7 years) and a median BSA of 1.74 (0.78 to 2.36) m^2 performed between December 2017 and September 2019 at 9 ACTION (Advanced Cardiac Therapies Improving Outcomes Network) centers. Dilated cardiomyopathy, dilated cardiomyopathy in neuromuscular disease (20%), and congenital heart disease (17%) were indications for implantation of ventricular assist devices in 63%.

The anticoagulant and antiplatelet therapy protocols had some differences due to the experience of the HeartMate III implantation center (Table 4).

As can be seen from Table 3, warfarin therapy in combination with aspirin was preferred and was used in almost the entire patient cohort. The authors showed that during the follow-up period (median follow-up 78 days) no complications such as acute cerebral circulation disorder, ventricular thrombosis and significant bleeding were reported [35].

Although the current standard anticoagulant therapy is most commonly given with vitamin K antagonist warfarin, this poses certain challenges associated with the need for frequent monitoring of the international normalized ratio, dietary compliance and repeated hos-

Table 3

Anticoagulant therapy protocols for long-term pediatric MCS

Protocol name	Early p/o period	Long-term p/o period	Antiplatelet agents	Monitoring	Other
Edmonton Protocol [28]	Heparin	Enoxaparin ≤12 months Warfarin >12 months	Dual antiplatelet therapy: Dipyridamole 4 mg/kg/day Aspirin 1 mg/kg/day	Thromboelastogram + platelet aggregation	
Stanford Protocol [29]	Heparin	Enoxaparin ≤12 months Warfarin >12 months	Triple antiplatelet therapy: Aspirin 30 mg/kg/day Dipyridamole 15 mg/kg/day Клопидогрел 1 mg/kg/day	None	Steroids in systemic inflammatory response
ACTION protocol [30]	Bivalirudin	Coumarin	Aspirin	Thromboelastogram + platelet aggregation	Steroids in systemic inflammatory response

Table 4

Combinations of anticoagulant and antiplatelet therapy for long-term MCS

Drug	n (%)
Unfractionated heparin	28 (77.8%)
Low-molecular-weight heparin	4 (1.1%)
Bivalirudin	8 (22.2%)
Warfarin	31 (86.1%)
Aspirin	34 (94.4%)
Clopidogrel	0
Dipyridamole	0

pitalizations associated with complications from sub- or over-therapeutic international normalized ratio values.

New direct-acting oral anticoagulants (direct thrombin inhibitors (dabigatran) or direct factor Xa inhibitors (rivaroxaban, apixaban, edoxaban and betrixaban) do not require laboratory monitoring. Potential disadvantages of these groups of drugs are their higher cost and shorter half-life compared with warfarin. This is associated with higher risk of thrombosis if the drug is missed. Currently, new direct-acting oral anticoagulants have begun to be considered as an alternative to vitamin K antagonists in the management of patients on long-term MCS systems.

The only randomized trial comparing the efficacy of phenprocoumon with dabigatran in patients after HeartWare ventricular implantation (HVAD) was terminated prematurely due to frequent thromboembolic complications in the group of patients taking dabigatran: 3 of 8 patients had ventricular thrombosis, one patient had transient ischemic attack [36].

The experience of using factor Xa inhibitors in this patient cohort is limited to single publications. For example, Parikh V. et al. reported seven patients who received anticoagulant therapy with rivaroxaban and apixaban in the postoperative period (one Heartmate II patient took rivaroxaban 15 mg daily, two HeartWare patients took rivaroxaban 15 mg daily, and four HeartWare patients took apixaban 5 mg twice daily). No thromboembolic complications were registered during the follow-up period of 1459 days, in 2 cases gastrointestinal bleeding developed in the late postoperative period (0.07 cases per patient per year) [37].

Schulte-Eistrup S. et al. reported on 22 patients after HeartWare implantation whose therapy was converted from vitamin K antagonists to apixoban at a dose of 5 mg daily in combination with aspirin (n = 10) or clopidogrel (n = 12) due to inability to regularly monitor the ongoing anticoagulant therapy. There were no fatal complications related to apixoban administration during the follow-up period of 408 ± 296 days (45–1554) [38].

In a retrospective randomized clinical trial, Song L et al. compared the incidence of stroke, bleeding, thrombotic complications, and death in patients after HeartMate III implantation by dividing them into two groups. The first group included 20 patients treated with warfarin; the second group (n = 15) received apixaban as anticoagulant therapy. During the 6-month follow-up period, there were no significant differences in complications and deaths between the two groups [39].

CONCLUSION

Peculiarities of postoperative management of children and adolescents after implantation of long-term MCS devices are crucial for stable operation of the ventricular assist device. Conscious anticoagulant and antiaggregant therapy, monitoring in the early and late postoperative period of the administered drug therapy, as well as control of laboratory parameters during conversion from one drug to another, can reduce the risks of bleeding and thromboembolic complications in this patient cohort.

Until recently, heparin and warfarin were used as the drugs of choice for anticoagulant therapy in patients on long-term mechanical circulatory support. With the emergence of new groups of anticoagulant drugs, interest in their use has increased significantly, which is associated with more convenient dose titration, absence of the need for regular monitoring of hemostasis parameters and low risks of postoperative complications against the background of ongoing treatment.

Muhammad Bakr Ghbeis et al. reported on their own experience of anticoagulant and antiplatelet therapy in the postoperative period in children requiring implantation of long-term MCS systems.

Examination before MCS implantation

1. ***Laboratory monitoring***
   a. *Mandatory examination:*
      – Determination of platelet count, prothrombin time, INR, aPTT, fibrinogen level, basic metabolic indicators
   b. *Additional examinations*
      – Thromboelastography with platelet mapping, C-reactive protein level, LDH, cystatin C, heparin-induced thrombocytopenia screening
2. ***Medical history and family history***
   – Bleeding and thrombosis history
      a. *With a burdened history, additionally:*
         – Anticardiolipin, beta-2 glycoprotein, lupus anticoagulant, factor V Leiden, prothrombin 20210A gene mutation, antithrombin 3 and protein S and C.

After MCS implantation:

1. ***Day zero after surgery***
   – After completion of hemostasis, bivalirudin administration at a dose of 0.1–0.3 mg/kg/h at an initial target aPTT of 50–60 seconds, with subsequent dose increases over several days until aPTT of 70 to 90 seconds is achieved.

2. ***First postoperative days***
- *Paracorporeal mechanical circulatory support*
  - Continuation of bivalirudin therapy. Start of antiplatelet agents within 1–2 weeks after implantation. If signs of fibrin formation or clot formation develop on the ventricular assist device, earlier initiation of antiplatelet therapy is possible.
- *Intracorporeal mechanical circulatory support*
  - Therapy with bivalirudin or unfractionated heparin in early stages with subsequent transition to indirect anticoagulants with maintenance of target INR of 2 to 3.5. On day 5 to day 7, antiplatelet agents should be added to the therapy.

The authors have shown effective use of this protocol in the management of children and adolescents after implantation of long-term MCS systems [40].

Changes in anticoagulant therapy strategy with the advent of the direct thrombin inhibitor bivalirudin, new oral direct-acting anticoagulants and direct Factor Xa inhibitors open up new perspectives in personalizing the approach in treating children implanted with long-term MCS systems.

The authors declare no conflict of interest.

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MID-TERM AND LONG-TERM OUTCOMES FOLLOWING HEART TRANSPLANTATION WITH PROLONGED COLD ISCHEMIA

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Objective: comparative analysis of long-term outcomes following heart transplantation (HT) with prolonged and short cold ischemia. **Materials and methods.** We analyzed the data of 29 orthotopic HT with >4 hours of cold ischemia. The transplant surgery was performed at Meshalkin National Medical Research Center between 2013 and the present time. Organs were obtained from donors from other regions. The control group consisted of 29 HTs with cold ischemia <4 hours, performed in the same period. The minimum distance between the transplant center and the donor base was about 250 km (Barnaul); the maximum distance was about 850 km (Krasnoyarsk). Recipient survival and postoperative peculiarities were analyzed. **Results.** In-hospital survival in the prolonged cold ischemia group was 89.7% (n = 26) with 3 deaths (10.3%). In the second group (<240 min), in-hospital survival was 79.3% (n = 23) with 6 (20.7%) deaths. The Kaplan-Meier survival analysis showed no difference between the groups (Log-Rank Test, P 1/4 0.59). In addition, cold ischemia time did not increase the risk of graft rejection and the risk of transplant coronary artery disease (TCAD). **Conclusion.** HT with cold ischemia >4 hours did not have worse outcomes than in short graft ischemia. This provides grounds for further accumulation of experience in the use of heart donors from remote locations.

Keywords: heart transplantation, cold ischemia time.

INTRODUCTION

Today, more and more people are in need of heart transplantation due to progression and decompensation of chronic heart failure (CHF) [1]. The low level of organ donation and critical shortage of donor pool remain a stumbling block for organ and tissue transplantation in Russia. Despite some recent improvements, Russia is still very far from the leading positions in the organ donation ranking. A large number of regions are not involved in the organ donation and transplantation program at all. The real level of development of organ donation and transplantology depends only on a few regions; in most of the rest, there are single organ transplants [2]. This necessitates the search for optimal use of unclaimed organs from regions with poorly developed organ donation. The use of organs from remote regions is related to a number of limiting factors associated with the difficulty of assessing the quality of the donor heart, longer transportation, logistics difficulties and, of course, prolonged ischemia time. It is well known that prolonged cardiac ischemia is accompanied by high risk of graft dysfunction and high mortality [3]. According to the Russian national HT guidelines, cold ischemia >4 hours is a risk factor for impaired myocardial function, and only young donors should be considered when using hearts with prolonged ischemia time [4]. In available publications, there is no consensus on a particular safe cold cardiac ischemia threshold; there is also no agreement on the criteria for selection of donors and recipients for longer transportation.

Currently, there are no clear criteria that would help to reliably say that longer cold ischemia time can lead to mortality; risk factors have not been studied. The paper presents an analysis of the experience of Meshalkin National Medical Research Center. The specifics of donor service involve constant interaction with neighboring regions and longer transportation of donor hearts.

MATERIALS AND METHODS

A retrospective analysis of 58 heart transplants performed between July 20, 2012 and October 23, 2019 was carried out. Patients were divided into two groups depending on cold ischemia time. The first group of the study consisted of 29 recipients who underwent orthotopic HT with cold ischemia time >240 minutes, the

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Table

Patient characteristics

Characteristics	Group 1 (>240 min)	Group 2 (<240 min)
Age, years	42 [33–51]	47 [36–50]
BMI (kg/m^2)	24.8 [23.3–31]	26.4 [22.4–29.5]
Gender		
Men	20 (69%)	27 (93.1%)
Women	9 (31%)	2 (6.9%)
Diagnosis		
DCM	25 (86.2%)	15 (51.7%)
ICM	3 (9.6%)	12 (37.9%)
HCM	0	1 (3.4%)
Rheumatism	0	1 (3.4%)
CHD	1 (3.4%)	0
Cold myocardial ischemia time, min	350 [300–390] (240–456)	165 [150–180] (135–240)
Urgency status (UNOS)		
1a	3 (10.3%)	2 (6.9%)
1b	5 (17.2%)	2 (6.9%)
2	21 (72.4%)	25 (86.2%)
LVAD	6 (20.6%)	3 (10.3%)
ECMO	4 (15.4%)	2 (6.9%)

second group – 29 recipients with short cold ischemia time (<240 minutes). Baseline data of the recipients are presented in Table.

Mechanical circulatory support (VAD) as bridge to transplantation was performed in 8 group 1 patients (27.6%) and 3 group 2 patients (9.6%). Extracorporeal membrane oxygenation (ECMO) as a bridge to OHT was performed in 4 group 1 patients (13.7%) and two group 2 patients.

Prolonged graft ischemia was as a result of longer transportation from neighboring regions: Kemerovo Oblast, Krasnoyarsk Krai, and Altai Krai. Transportation from Kemerovo Oblast and Altai Krai was on official vehicles (cars); Transportation from Krasnoyarsk Krai was by civil aviation.

Selection criteria for postmortem donors were standard. In all cases, single-group transplants were performed taking into account donor/recipient matching by constitution. The gender distribution of donors did not differ significantly. Donors with inotropic support >20 mg/kg/min (dopamine or dobutamine) or similar doses of other adrenergic drugs, despite aggressive optimization of pre- and postload, were not considered. The median age of donors in the prolonged ischemic group was 40 (34–46) years, in the short ischemic group 43 (40–51) years. Criteria for donor heart evaluation were standard, expanded criteria donor were not considered. The harvesting technique and method of heart preservation were standard.

A bicaval approach was used for OHT. To assess volumetric characteristics of the recipient heart after transplantation and pulmonary artery pressure, all patients underwent follow-up transthoracic echocardiography (EchoCG) immediately after surgery, at day 5–10, and month 1 after surgery. The risk of donor heart rejection was also analyzed based on endomyocardial biopsy results according to the recommended ISHLT WF 2004 classification (International Society for Heart and Lung Transplantation – working formulation, 2004).

Overall recipient survival was taken as the primary endpoint. Maximum follow-up period was 137 weeks in the long graft ischemia group and 124 weeks in the short graft ischemia group. Inotropic index values at the time of disconnection from the heart-lung machine, differences in the frequency of graft dysfunction and the need for mechanical circulatory support in the perioperative period, graft rejection, as well as risk factors of postoperative complications were also analyzed.

Taking into account small sample size and non-normal distribution (according to Shapiro–Wilk test), data were presented as median, 1st, 3rd quartile. Nonparametric statistical criteria were used: Mann–Whitney U test for comparison of independent samples. Single-factor regression analysis was used to identify predictors of mortality.

STUDY RESULTS

Duration of inotropic support

Primary graft dysfunction and the need for continued mechanical circulatory support in the postoperative period was observed for only 4 patients (15.4%) in the prolonged graft ischemia group and 3 patients (11.5%) in the short cold ischemia group (Fig. 1). These patients underwent peripheral ECMO cannulation. The level of inotropic support at the time of circulatory arrest was represented by the inotropic index, with no significant difference between the two groups (p = 0.13) (Fig. 2). Median inotropic index was 8 (4–14.75) in group 1 and

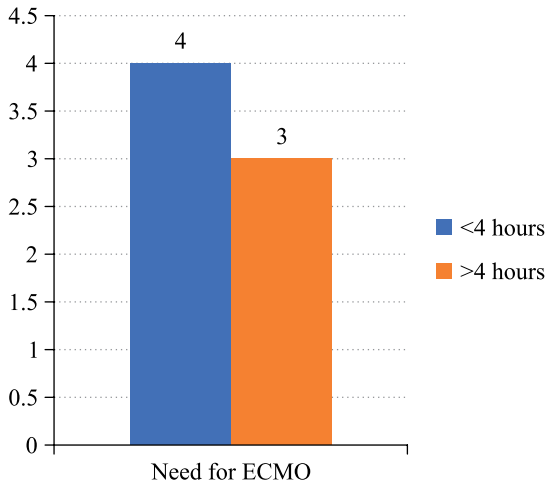

Fig. 1. Need for ECMO in the postoperative period in the study groups

6.75 (3.25–8) in group 2. Total duration of inotropic support was slightly longer in the prolonged graft ischemia group ($p < 0.05$) (Fig. 3).

In-hospital survival in the prolonged cold ischemia group was 89.7% (n = 26) with 3 deaths (10.3%). Mortality structure in the prolonged cold ischemia group was as follows: 1 patient died from primary graft dysfunction and 2 patients died from initial severe condition (desperate surgery), IA(UNOS) (Fig. 4). In one case, death was caused by severe right ventricular failure after switching off ECMO. In the other case, intraoperative massive diffuse bleeding against coagulopathy, which was caused by extremely severe pre-transplant condition of the recipient. In the second group (<240 minutes), in-hospital survival was 79.3% (n = 23) with 6 (20.7%) deaths, respectively. The mortality structure is shown in Fig. 5. The causes of mortality were acute rejection, graft dysfunction, ischemic stroke, hospital-acquired pneumonia, and tricuspid regurgitation (TR). Thus, 1 patient in each group died from primary graft dysfunction in the early postoperative period. The Kaplan–Meier survival analysis showed no difference between the groups (Log-Rank Test, P 1/4 0.59) (Fig. 6).

In the long-term postoperative period, 3 patients from the short graft ischemia group died of unknown causes, 1 died due to severe TR after iatrogenic damage to the tricuspid valve chordal apparatus during myocardial biopsy, 2 patients died due to severe coronavirus infection, 1 died from abdominal infection (Fig. 5). In the prolonged cold ischemia group, 1 patient died of unknown causes, 1 died from progression of cancer at month 1 and 3, respectively. In the mid-term and long-term, 1 patient died from cancer, 4 patients died from COVID-19.

In the mid- and long-term period, significant TCAD was detected in 3 patients from the prolonged ischemia

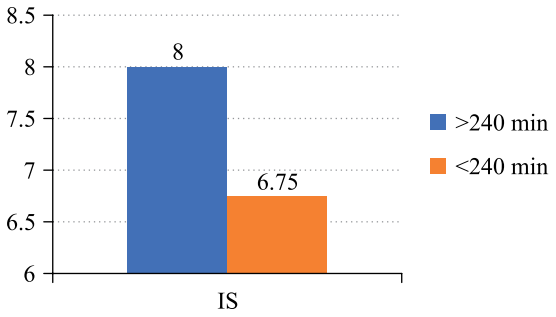

Fig. 2. Inotropic score in the study groups

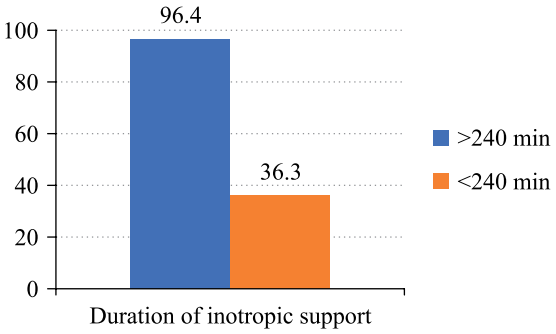

Fig. 3. Duration of inotropic support in the study groups

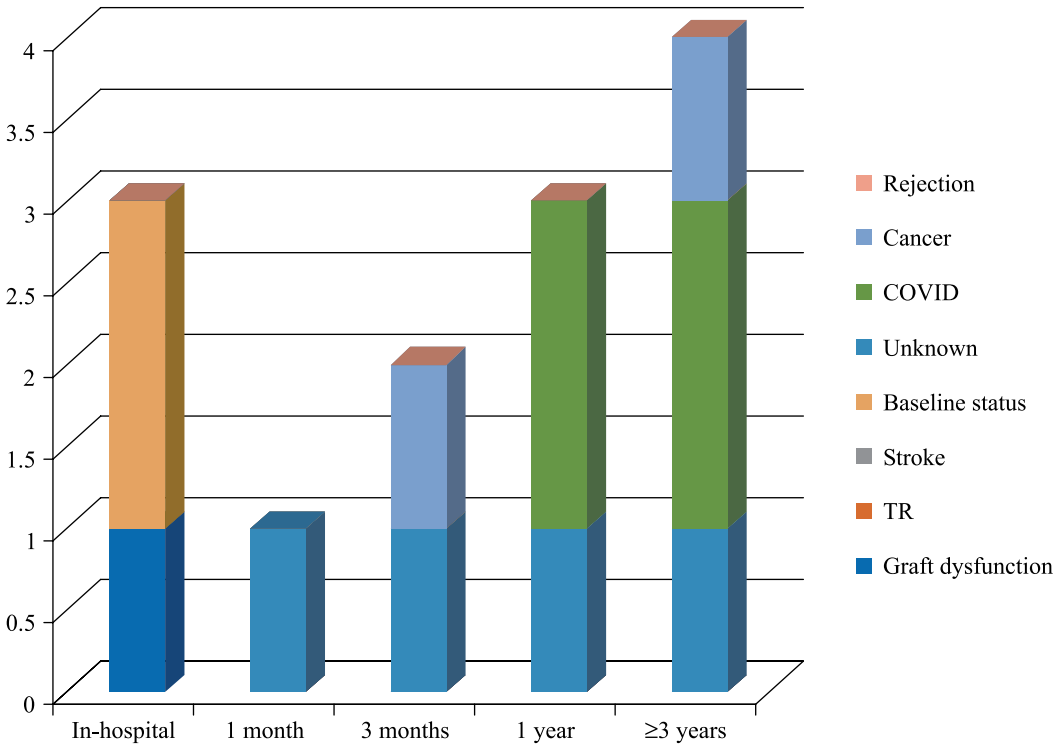

Fig. 4. Mortality patterns in the prolonged graft ischemia group

group and in 6 patients from the short ischemia group (Fig. 7).

When analyzing the frequency and degree of acute cellular rejection in the study groups in the first 30 days after transplantation based on the classification of the International Society for Heart and Lung Transplantation (ISHLT, 2004), it was shown that cold ischemia time had no adverse effect on HT outcomes in the 30-day follow-up period. The study groups of recipients were characterized by a dominant mild rejection (G1R), which did not require radical adjustment of immunosuppressive therapy.

DISCUSSION

Despite active development of perfusion technologies and attempts to optimize the organ donor pool, the issue of the use of expanded criteria heart donors and, in particular, hearts with prolonged cold ischemia, remains relevant [5].

Given the fact that in a number of cases, donor hearts from expanded criteria donors were implanted in urgent recipients after longer transportation, the initial severity of patients in the study group was slightly higher than in the comparison group. Two deaths were related specifically to the terminal state of the recipients, for whom HT

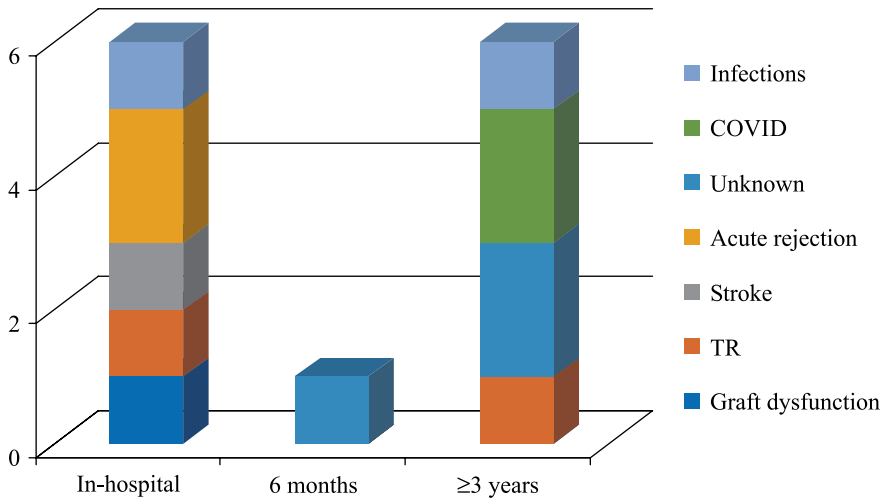

Fig. 5. Mortality patterns in the short graft ischemia group

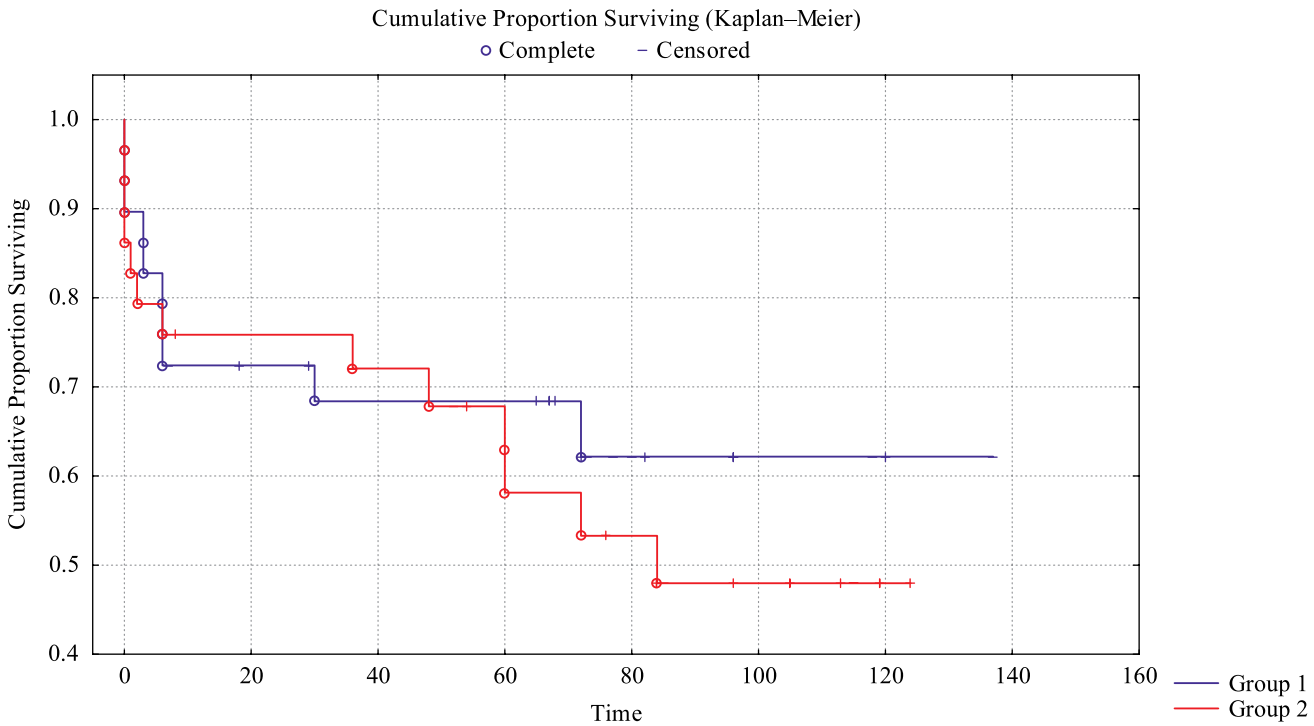

Fig. 6. Recipient survival for the entire follow-up period (months). Log-Rank Test (Spreadsheet1) WW = –1.376 Sum = 21.231 Var = 5.4010 Test statistic = –0.592046 p = 0.55382

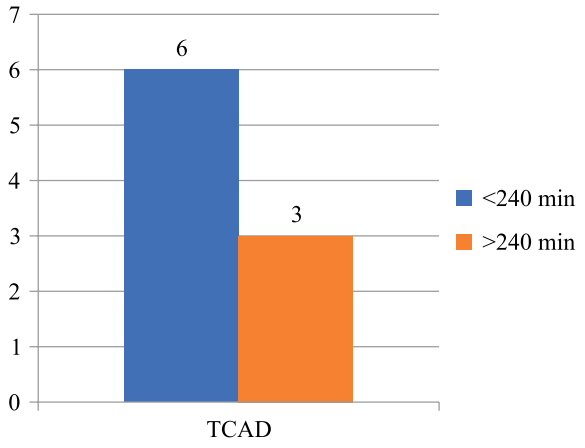

Fig. 7. Transplant coronary artery disease in the study groups

was performed as a desperate surgery. However, it did not lead to higher mortality in the prolonged ischemia group. Some authors single out prior mechanical support as a risk factor for adverse outcome [6].

Undoubtedly, modern realities contributed to the course of the post-transplant period. A total of 6 people in both groups died due to COVID-19 complications. Despite the fact that 4 people died from COVID-19 in the prolonged ischemia group and two in the comparison group, we did not obtain a significant difference in recipient survival in the groups. Analysis of one of the main potential negative factors of prolonged ischemia, primary graft dysfunction requiring mechanical circulatory support, also showed no advantage of short graft ischemia.

One of the identified consequences of the negative impact of prolonged donor heart ischemia was the duration and higher doses of inotropic support in the early postoperative period.

Unfortunately, we were unable to determine the threshold of safe cold ischemia time, probably because of the small sample of observations.

Available reports contain a lot of data on the adverse effect of cold ischemia >240 minutes on transplant outcomes. Similar results were obtained by authors from Spain, who analyzed HT outcomes in 17 centers in Spain for 2008–2018. They concluded that cold ischemia >4 hours has an adverse effect on 1-month and 1-year mortality [5].

In analyzing 317 heart transplant outcomes, a group of authors from the United States determined that each hour of cold ischemia increases the risk of primary graft dysfunction 1.8-fold [6].

Undoubtedly, there are a number of reports showing that HT with graft ischemia >300 minutes or more is possible.

Scientists from the USA have established that HT with cold ischemia >5 hours is accompanied by worse survival in the presence of such risk factors, ECMO and dialysis in the preoperative period, diagnosis of ischemic cardiomyopathy, as well as when using group 0(I) donors [7].

Authors from Botkin Hospital and the Shumakov National Medical Research Center of Transplantology and Artificial Organs distinguish between optimal (<180 minutes) and prolonged (>240 minutes) cold ischemia time. The threshold value is considered to be 300 minutes; in the presence of risk factors (advanced age, high doses of inotropic support, etc.), this threshold should not be exceeded [8].

Through analysis of 323 HT outcomes, the safe cold ischemia threshold for HT was established at ≤300 minutes [9].

An interesting analysis of the outcomes of 25,996 HTs (UNOS) was performed by authors from the USA. Patients were stratified by distance between the transplantation center and the donor base. 134 transplants were performed with a long transport of more than 1,500 miles and cold ischemia time of 7.5 hours. There was no significant difference in 1-year and 5-year survival between the groups [10].

Analysis of literature data is hampered by the heterogeneity of studies – different geography, principles of organ distribution, peculiarities of individual centers, and experience.

Despite the external factors influencing data analysis, as experience is gained, taking into account the peculiarities of the organ donation system in Siberian Federal District, the immediate and long-term outcomes of HT with prolonged cold ischemia are comparable with HT outcomes with ischemia <240 minutes. Obviously, the use of donor organs with prolonged cold ischemia has its own inclusion and exclusion criteria and risk factors. However, a clear algorithm for using this donor category, which will take into account all possible features of the regions and individual transplantation centers, is required.

CONCLUSION

HT with cold ischemia >4 hours did not show worse HT outcomes than short graft ischemia. Given the small experience, we were not able to identify the ischemic threshold and mortality predictors. However, survival after heart transplantation with prolonged cold ischemia provides grounds for further accumulation of experience in the use of heart donors from remote regions.

The authors declare no conflict of interest.

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HEMODYNAMIC EVALUATION OF PULSATILE-FLOW GENERATING DEVICE IN LEFT VENTRICULAR ASSIST DEVICES

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Objective: to investigate the efficiency of a device that generates pulsatile flow during constant-speed axial-flow pump operation for use in left ventricular assist devices. **Materials and methods.** The pulsatile flow-generating device, hereinafter referred to as "pulsator", consists of a variable hydraulic resistance made in the form of a hull. A tube of elastic biocompatible material featuring an inner diameter of 11 mm is installed inside it. In the systolic phase of the left ventricle, due to systolic pressure, the elastic tube is fully opened, minimizing resistance to blood ejection. In the diastolic phase, due to suction action of the flow pump operating in constant revolutions, the elastic tube partially closes, creating additional hydraulic resistance to blood flow, which leads to reduced diastolic aortic pressure. Comparative assessment of axial-flow pump operation in pulsating and non-pulsating modes was carried out on a hydrodynamic stand that simulated the cardiovascular system. The following indices were calculated: arterial pressure pulsation (I_p), in-pump flow pulsation (ΔQ), energy equivalent pressure (EEP) and surplus hemodynamic energy (SHE). **Results.** When comparing axial-flow pump operation in pulsatile and continuous mode, arterial pressure pulsation index, in-pump pulsation index, and SHE index increased by 2.13 ± 0.2, 3.2 ± 0.2, and 2.7 ± 0.15 times, respectively, while EER index remained unchanged.

Keywords: heart failure, left ventricular assist devices, continuous flow, pulsatile flow, hydrodynamic stand, axial-flow pump.

INTRODUCTION

In clinical practice, continuous-flow circulatory assist devices have replaced pulsatile-flow circulatory assist devices, when applied as a bridge to transplantation and targeted therapy. This is due to a number of advantages of continuous-flow pumps (CFPs) over pulsatile-flow pumps (PFPs): they are placed inside the thoracic cavity, have small weight-and-size characteristics, better power consumption and performance characteristics. It promoted an increase in the number of various CFP models and their mass distribution, which led to an increase in these devices and considerable increase in survival rate among patients with end-stage heart failure (HF) [1–3]. However, when studying a large number of CFPs in conditions of long-term use, a number of complications, which were a consequence of low arterial pulsation, were revealed. These include gastrointestinal bleeding, arteriovenous malformation, aortic insufficiency, etc. [4–6].

In connection with the search for solutions to reduce complications, a number of works have shown the need to increase the level of pulsatile flow not only in extracorporeal systems, but also in implantable systems, including left and right ventricular assist devices (LVAD and RVAD) [7–9]. In the last decade, many researchers have mostly focused on developing flow pulse wave enhancement methods using the principle of electrocardiogram (ECG)-synchronized modulation of pump velocity [10–14]. The main disadvantage of this method is inertia of most circulatory assist pumps, which did not allow to obtain a given arterial pulsation especially when the heart rate (HR) was increased. Besides, there are still open questions related to the level of hemolysis in the blood in the CFP speed modulation mode [15].

To increase the level of pulsation in CFPs, we earlier proposed a method using parallel connection to the pump (input–output) of the recirculation channel [16–17]. In this case, the system operation was provided by a controlled electromechanical valve installed in the recirculation channel, which required relatively high power to close the recirculation channel in the systolic phase [18].

The present work shows a more efficient version of the device for enhancing pulsatile flow in CFPs, which has many potential advantages, the main one being realization of self-sustainable operation (without external control sources and heart rhythm signals) and formation of ECG-synchronized pulsatile flow and pressure.

MATERIALS AND METHODS

Pulsatile flow enhancement is based on connection to the CFP inlet line of the pulsator (Fig. 1) made in the form of a hull (1) with a tube of elastic biocompatible

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material (polyurethane) with inner diameter of 11 mm (2) installed inside it. An air cavity (4) is formed between the body (1) and the tube (2), which communicates with the atmosphere through the holes (3) in the hull (1) (Fig. 1, a).

The pulsator works in the following way: in systolic phase, due to left ventricular (LV) pressure and CFP work, the elastic tube opens fully, reducing pressure drop inside the elastic tube (2), and forming maximum flow and pressure amplitude.

In diastolic phase, when LV pressure and CFP suction action decrease, pressure inside elastic tube (2) decreases, resulting in partial closure of elastic tube and an increase in hydraulic resistance to flow from the LV to the CFP, which leads to a decrease in arterial diastolic pressure. Thus, an ECG-synchronized pulsatile flow and pressure (co-pulsation mode) is formed at the CFP outlet.

At the first stage of the research, we assessed the efficiency of the pulsator on a hydrodynamic stand (HS) when connecting an axial pump in the left ventricular assist mode of the heart. The design of the HS used was previously described in Buchnev A.S. et al. [16]. Fig. 2 shows the general view of the HS, which consists of an axial-flow CFP (1) – a portable auxiliary circulatory assist device (AVK-N, Russia), pulsator (2) installed in the inlet line (3) of the CFP. A Medos 80 mL pulsatile pump (Medos, Germany) with pneumatic drive SINUS-IS (MZEMA, Russia) was used as the LV heart simulator (5). CFP outlet (4) was connected to the aorta, designed as a reservoir with an air cushion (6) associated with systemic peripheral resistance (10) and venous reservoir

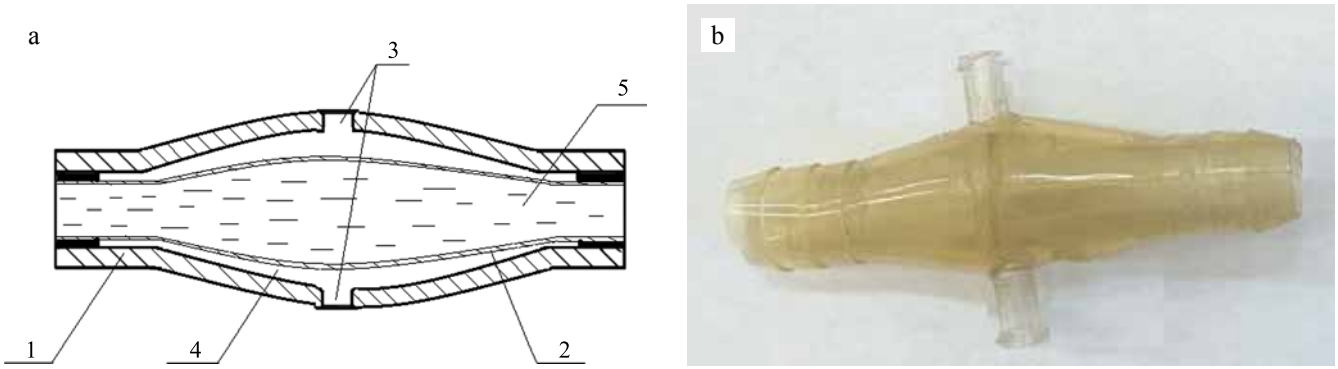

Fig. 1. a, schematic diagram of pulsator (1, hull; 2, elastic tube; 3, holes; 4, air cavity; 5, flow path); b, external view of pulsator

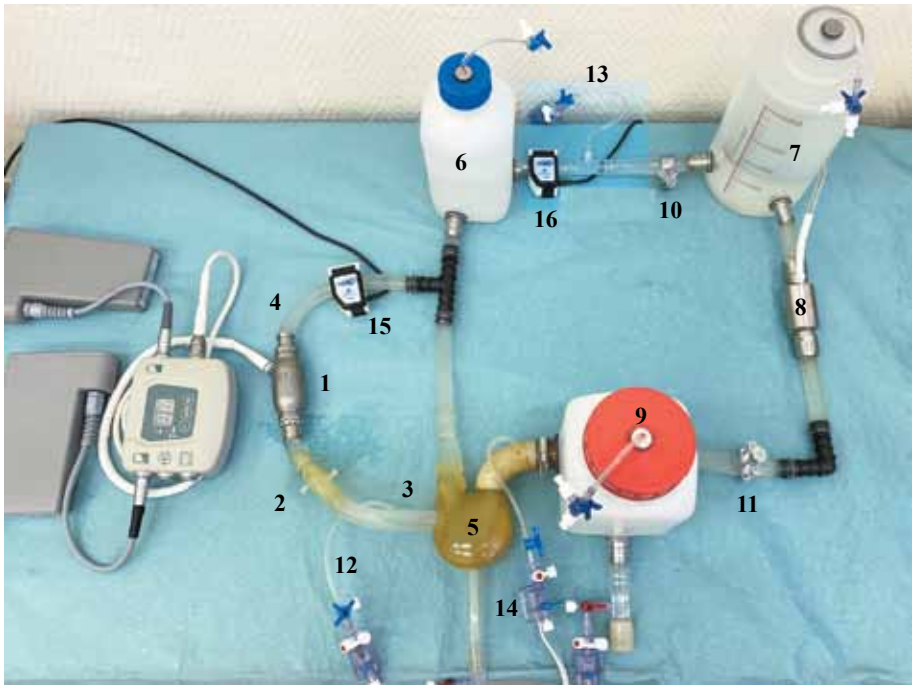

Fig. 2. Hydrodynamic stand: 1, non-pulsatile flow pump (AVK-N); 2, pulsator; 3, inlet cannula; 4, outlet cannula; 5, artificial heart ventricles; 6, aortic reservoir; 7, venous reservoir; 8, non-pulsatile flow pump (VISH); 9, left atrium; 10, systemic hydraulic resistance; 11, pulmonary resistance; 12–14, pressure sensors; 15, 16, flow sensors

(7). As the right ventricle of the heart, a mockup sample of implantable axial-flow pump VISH (Russia) was used (8). The flow in the pump and aorta was measured using ultrasonic flowmeter T402 (Transonic Systems Inc., USA) (15, 16). Pressures in LV, aorta, and left atrium were measured using pressure transducers (Edwards Life, USA) (12–14). We preliminarily simulated normal conditions on hydrodynamic stand, which were set by changes in LV pneumatic pressure, parameters of aortic capacity, systemic and pulmonary resistance in accordance with the Pantalos et al. guidelines [19], systemic flow was 5.0 ± 0.2 L/min, arterial pressure 118/81 ± 5 mm Hg. To record hemodynamic parameters, we used the multi-channel module Angioton (Biosoft-M, Russia) with recording on a personal computer in Pumpax program (Biosoft-M, Russia).

Heart failure mode was set by changes in LV cardiac pressure and systemic peripheral resistance without changes in aortic capacity. The following parameters were set: mean aortic flow 2.7 ± 0.3 L/min and aortic pressure 80/55 ± 5 mm Hg. When CFP was turned on, the pressure in the artificial ventricle was set at 60 ± 5 mm Hg (Frank-Starling Law). After that, continuous-flow and pulsatile-flow pump operation modes were started.

The aortic pulsation obtained during the experiments was analyzed based on the pulsatility index (Ip) calculated by the formula:

$$I_p = (P_{ao(max)} - P_{ao(min)}) / P_{ao(avr)}, \quad (1)$$

where $P_{ao(max)}$ is arterial systolic pressure, $P_{ao(min)}$ is arterial diastolic pressure and $P_{ao(avr)}$ is mean arterial pressure.

Surplus hemodynamic energy (SHE) was calculated using the Shepard equation [20]:

$$SHE(ergs/cm^3) = 1332 \times (EEP - P_{ao(avr)}), \quad (2)$$

where the energy equivalent pressure (EEP) was calculated by the formula:

$$EEP(mmHg) = \int_{t1}^{t2} fp\,dt \,/ \int_{t1}^{t2} f\,dt, \quad (3)$$

where f(t) is the aortic flow time curve for a fixed period of time, p(t) is the aortic pressure time curve for the same time period.

STUDY RESULTS

Fig. 3 shows the hemodynamic parameters taken on a HS in simulations of physiological norm (a) and heart failure (b). In the norm, systemic flow rate was 5.0 ± 0.2 L/min and blood pressure 118/81 ± 5 mm Hg. In HF, systemic flow rate was reduced to 2.7 ± 0.2 L/min and blood pressure to 80/55 ± 5 mm Hg.

Fig. 4 (a, b) shows hydrodynamic parameters when the pump is operating in continuous (n = 9200 rpm) and pulsatile modes (n = 10,000 rpm). At the same time,

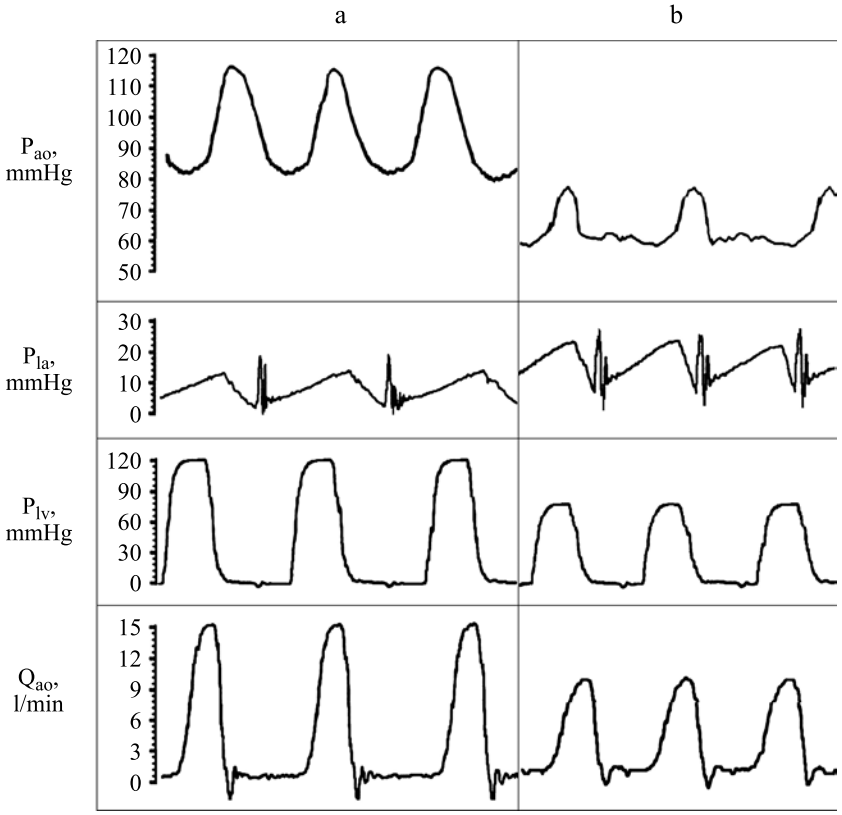

Fig. 3. Comparative results of hemodynamic parameters in norm (a) and in heart failure (b). (P_{ao}, arterial pressure; P_{la}, left atrial pressure; P_{lv}, left ventricular pressure; Q_{ao}, systemic blood flow)

pump speed during pulsator operation was increased to maintain mean arterial pressure and systemic flow rate at the same level as during continuous operation.

Hemodynamic parameters during CFP operation in continuous mode: systemic flow rate (5.0 ± 0.2 l/min), arterial pressure (95 ± 5 mm Hg), arterial pressure pulsation (14 ± 1 mm Hg) and in-pump flow pulsation 3.0 ± 0.2 l/min (Fig. 4, a). Hemodynamic parameters during CFP operation with pulsator: systemic mean flow rate 5.0 ± 0.2 l/min and mean arterial pressure 95 ± 5 mm Hg. At the same time, aortic pulsation increased to 30 ± 5 mm Hg, and in-pump flow pulsation was 9.5 ± 0.2 l/min (Fig. 4, b).

Table shows the comparative results of the main hydrodynamic indicators and the Ip, EEP and SHE indices for pump operation in continuous-flow and pulsatile-flow modes.

Thus, the Ip index during CFP operation with a pulsator increased 2-fold compared to the continuous mode,

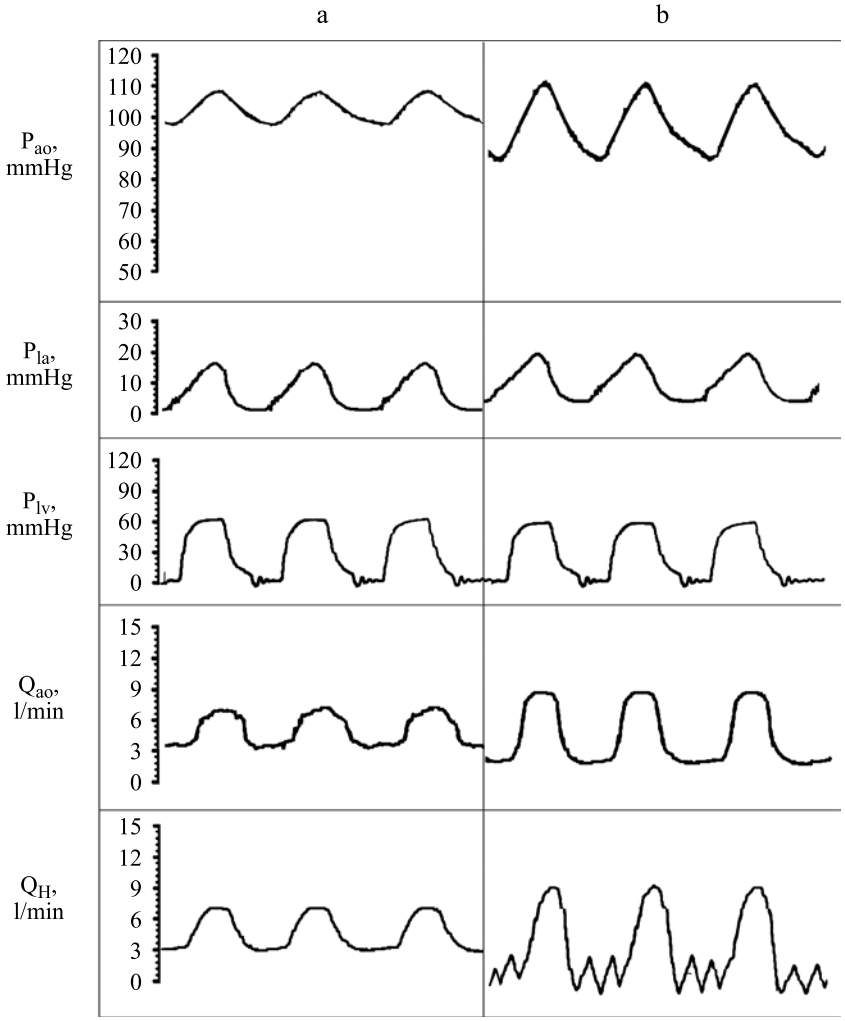

Fig. 4. Comparative results of hemodynamic parameters under conditions of continuous (a) and pulsatile (b) flows for left ventricular assist device (P_{ao}, arterial pressure; P_{la}, left atrial pressure; P_{lv}, left ventricular pressure; Q_{ao}, systemic blood flow; Q_H, flow through the AVK-N pump)

Table

Hemodynamic parameters of the CF-LVAD operating modes

	P_{ao} (mmHg)	Q_{ao} (l/min)	ΔQ_H (l/min)	I_p	EEP (mmHg)	SHE (ergs/cm^3)
Norm	118/81 (95)	20.2/0.2 (5.0)	–	0.38	99.44	5918
Heart failure	80/54 (65)	10.1/0.1 (2.7)	–	0.40	67.7	3596
Continuous mode	104/90 (95)	7.5/3.4 (4.9)	3.0	0.15	96.2	1599
Pulsatile mode	111/81 (95)	9.0/2.2 (5.1)	9.6	0.32	98.3	4393

P_{ao}, arterial pressure; Q_{ao}, aortic flow pulsation; ΔQ_H, pump flow pulsation; I_p, pulsatility index; EEP, energy equivalent pressure, SHE, surplus hemodynamic energy.

ΔQ increased 3.2-fold, the SHE index increased 2.7-fold, and the EER index remained unchanged.

DISCUSSION

The proposed method for increasing the flow pulsation level in CFP pumps is based on inclusion of a self-sustainable pulsator in the input line. The results of bench studies on a hydrodynamic stand showed the high efficiency of the method with simple design solutions in the implementation of this system. The pulsator works on a self-sufficiency basis without external energy control and feedback, closed on the electrical signals of the heart rate. With an increase in systemic blood flow against the background of CFP, rarefaction occurs in the left ventricle in the diastolic phase [21]. In this method, due to increased hydraulic resistance of the pulsator at the left ventricular outlet, rarefaction magnitude is significantly reduced. The pulsatile mode of the CFP in diastolic phase minimizes blood flow from the LV, which leads to more complete LV filling and according to Frank-Starling Law to subsequent more complete LV ejection. This device provides high flow pulsation, comparable with physiological parameters of blood circulation, which helps to reduce formation of stagnation zones and flow recirculation in CFP. This will help to reduce the chances of thrombosis both in the pump itself and in the inlet cannula of the circulatory assist system.

CONCLUSION

This paper demonstrates the first stage of the study of a pulsatile flow enhancement device, which can be considered as an effective method of increasing arterial pulsation in LVADs. Further studies will include optimization of the device dimensions for implanted auxiliary circulatory assist systems using a helium-filled compensation chamber. Comparative hemolysis studies of the device and optimization of the pulsator design for right ventricular bypass are envisaged. In the future, we consider the possibility of developing a pediatric system of pulsatile flow enhancement and biventricular bypass using this device, and application in extracorporeal membrane oxygenation systems.

The authors declare no conflict of interest.

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BIOLOGICAL AND FUNCTIONAL PROPERTIES OF HUMAN UMBILICAL CORD-DERIVED LYOPHILIZED TISSUE-ENGINEERED MATRICES

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The use of tissue-engineered products (TEP) from decellularized extracellular matrix (dECM) to treat deep skin lesions is a tissue engineering method that promotes regenerative healing. Cell-free preparations reproduce the hierarchical complexity of tissues, mimic structural, biochemical and mechanical signals that are necessary to attract cells, and are a source of bioactive molecules. The human umbilical cord biomaterial has a fetal phenotype with extra-embryonic origin, and therefore is available and has no ethical limitations in its use. The tissue engineering laboratory at Kirov Military Medical Academy developed and patented a TEP from the highly regenerative human umbilical cord in the form of matrix and hydrogel matrix. To study its regenerative potential, lyophilisates of tissue-engineered solid-state and hydrogel matrices were implanted around mini pig full-thickness wounds in vivo. The external signs of inflammatory response and the histological images of biopsy specimens from the lyophilizate implantation areas were analyzed. The effect of nutrient media, "conditioned" with lyophilizates of both matrices, on the viability and migration activity of fibroblast-like cells, isolated from mini pig skin, was investigated. The matrix lyophilisates showed good biocompatibility and bioactivity in in vitro and in vivo experiments. Implantation of the samples promoted faster formation of mature epidermis compared to the control.

Keywords: tissue engineering, solid-state matrix, hydrogel matrix, human umbilical cord, full-thickness wound.

INTRODUCTION

Skin wound healing is a process of morphogenetic response to injury aimed at restoring the anatomical integrity and physiological function of the injured area [1]. Early and effective epithelialization of the wound defect with mature neoepithelium is required to restore the protective function of the skin [2]. The strategy of applying tissue-engineered products (TEP) from decellularized extracellular matrix (ECM) allows not only to accelerate granulation tissue formation by mechanical filling of the defect, but also promotes healing. The component composition, porosity and biodegradability of TEP serve as factors of biointegration and modulation of the local immune response. At the same time, the characteristics of the initial biomaterial for TEP fabrication, the procedure of cellular material removal and the final consumer form of the product affect its biological effectiveness [3]. The limitations of autografts, allogeneic and xenogeneic materials encourage researchers to look for alternative biomaterials [4].

The unique composition, homologous extra-embryonic origin, availability without invasive procedures and ethical restrictions make the human umbilical cord an attractive biomaterial for creation of highly regenerative TEPs. TEP (which can be manufactured in various forms) from a highly regenerative human umbilical cord was developed and patented in the tissue engineering laboratory of Kirov Military Medical Academy.

Objective: to evaluate the effect of the developed lyophilized TEPs (tissue-engineered solid-state and hydrogel matrices) from highly regenerative human umbilical cord biomaterial on skin cells in vitro and healing of full skin wounds of mini pig.

MATERIALS AND METHODS

Fabrication of cell-free tissue-engineered products from human umbilical cord

Human umbilical cords were obtained from healthy full-term newborns after spontaneous delivery with informed consent of the mothers and using guidelines approved by the Ethical Committee at Kirov Military Medical Academy, Protocol #203 (St. Petersburg, Russia).

To obtain a tissue-engineered ***solid-state matrix***, umbilical cord vessels were carefully removed under sterile conditions. Wharton's jelly was crushed with a blender (Bosch, Germany) and homogenized (gentle MACSTM Dissociator Milteniy Biotech, Germany), h-cord-01-01 program. Cells were removed using 0.05% sodium dodecyl sulfate solution (Biolot, Russia) for 24 hours at room temperature in a shaker at 140 rpm (Biosan, Latvia). Sodium dodecyl sulfate was removed by washing with phosphate-buffered saline (Biolot, Russia).

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To make the ***tissue-engineered hydrogel matrix***, 10 mg of dry tissue-engineered matrix was solubilized with pepsin solution (P/1120/46 Thermo Fisher Scientific, Germany) at the rate of 1 mg of enzyme in 1 ml 0.01 N HCl, pH 2.0, for 72 hours at room temperature and 180 rpm shaking. Pepsin was neutralized with 0.1 n NaOH solution to pH 7.4.

The resulting tissue-engineered solid-state and hydrogel matrices were lyophilized (ZirbusVaCo5II, Germany), sterilized by ultraviolet light in a LAMSYSTEMS microbiological safety box (Russia) with a UV-C radiation flux of 12 W for 15 minutes, and stored hermetically sealed at a temperature of –20 °C for a year.

Study of the dynamics of healing of full-thickness wounds in a mini pig

All manipulations with the animal were carried out in accordance with the ethical principles approved by the Ethics Committee at Kirov Military Medical Academy, Protocol No. 263 (St. Petersburg, Russia) and established by the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes [6]. A male mini pig No. 43-40 weighing 22.5 kg at the beginning of the experiment was obtained from the nursery of the Institute of Cytology and Genetics of the Siberian Branch of the Russian Academy of Sciences.

Zoletil 100 (Virbac, France, 10 mg/kg) and Xylazine (Xyla, Estonia, 0.1 ml/kg) were used intramuscularly for anesthesia. After careful removal of the hair on the back and treatment of the skin with 70% ethyl alcohol solution, DMP08 8-mm dermopunch (Sterylab, Italy) was applied five times at 7-day intervals in two full-thickness skin defects, 1 cm deep. 0.01 g of tissue-engineered solid matrix lyophilisate was placed into the experimental wounds. Observation of animal behavior, local skin reactions were recorded daily throughout the experiment, photographing the wounds with a scale bar in the calibration frame.

After 35 days, biopsies were taken from all experimental and control wounds. Then, two weeks later, the experiment was repeated, but 0.01 g of tissue-engineered hydrogel matrix lyophilisate was introduced into the wounds. Biopsy material was extracted, and the wounds were photographed on similar dates. Biopsy material was fixed in neutral formalin for 24 hours, embedded in paraffin, and histological sections were prepared and stained using Van Gieson's and Heidenhain's stains (Biovitrum, Russia) according to the manufacturer's instructions. The preparations were analyzed using an Axio Imager 2M microscope (Carl Zeiss, Germany).

Histological assessment of acute inflammation (HAAI) was performed at 1 and 2 weeks on a scale of 0 to 15. The following inflammation criteria were assessed: amount of neutrophilic cell infiltration in the dermis (0–3 points); amount of neutrophilic cell infiltration in the hypodermis (0–3 points); edema (0–3 points); bleeding (0–3 points); necrosis (0–3 points). The criteria used to determine the number of cellular infiltrates were as follows: 0, within normal histological limits; 1, scattered; 2, grouped or knotty; 3, diffuse. Histological assessment of edema: 0, no; 1, focal; 2, local; 3, diffuse. Degree of hemorrhage: 0, no; 1, soft; 2, moderate; 3, severe. Volume of necrosis: 0, no; 1, focal; 2, nodal/regional; 3, diffuse [2].

Quantitative data were calculated using ImageJ software. The relative area of the wound defect, the relative area of the vascular bed, the average vessel diameter, and epithelial thickness were determined. To calculate each morphometric index, 5 visual fields in each slice were examined, the total number of measurements n = 10.

Extraction and scaling of fibroblast-like mini pig skin cells

Skin fragments isolated for the production of full-thickness wounds, after removal of the fat layer, were washed three times with saline solution with antibiotics (penicillin, streptomycin for cell cultures, Biolot, Russia). Next, skin fragments were chopped with sterile scissors, placed in 25 cm^2 culture vials (Thermo scientific, USA), and 5 ml of nutrient medium was added. Culture medium DMEM (Biolot, Russia), 10% fetal calf serum (HyClone, USA), gentamicin for cell cultures 100 IU/ml (Biolot, Russia). After obtaining the primary mixed population of fibroblast-like cells, they were subcultured every 7 days at a 1 : 5 dilution in 25 cm^2 culture vials. Cellular material was detached from plastic by 0.02% trypsin-Versene solution (Biolot, Russia) in a 1 : 3 ratio at a temperature 37 °C for 5–7 minutes. After trypsin inactivation with nutrient medium and fetal bovine serum, cell material was precipitated by centrifugation at 3500 rpm for 5 minutes. The cell material was washed twice with nutrient medium under the same conditions. After the supernatant was removed, the precipitate was resuspended, and the cells were counted in a Goryaev chamber according to the conventional procedure.

Evaluation of the effect of lyophilized TEPs on the metabolic activity of mini pig skin cells

Fibroblast-like mini pig skin cells of the 4th passage were cultured in 96-well plates (Sigma-Aldrich, USA), 150 µl at a concentration of 13,000 cells per well. To study the effect of lyophilisates of both matrices on cell viability, conditioned nutrient media obtained by preincubation in the nutrient medium of the above composition of the studied TEP samples (1 mg/ml 24 hours at 4 °C to maintain sterility) were used. The study of cytotoxicity is mainly aimed at studying the effect of soluble substances contained in TEP, including sodium

dodecyl sulfate used for decellularization. The choice of 1 mg/ml concentration was guided by the intended amount and method of clinical application of TEP.

A day after cell adhesion to the surface of the culture plate, the initial medium was replaced with a conditioned medium and the control wells with a standard culture medium (n = 32). The plate was incubated for another 24 hours at 37 °C in 5% CO_2. Then, resazurin dye solution (Biocompass-S, Russia) was added to each well with 11.2 μM/ml as final concentration in the well in a volume equal to 10% of the total volume of culture liquid. Resazurin dye was dissolved in phosphate-buffered saline (PBS), pH 7.4 (Biolot, Russia) before adding it to the well. After 4 hours of incubation and shaking (2 min, 37 °C) on an orbital shaker (Biosan, Latvia), fluorescence levels were measured on a Victor X5 microplate reader (Perkin Elmer, USA) at 590 nm wavelength using an excitation wavelength of 560 nm. The results were expressed as a percentage relative to the control.

Evaluation of the migration activity of mini pig skin cells

Fibroblast-like mini pig skin cells of the 4th passage were cultured in 12-well plates (Sigma-Aldrich, USA) by 500 μl in concentrations of 45,000 cells per well. To study the effect of matrix lyophilisate samples on cell proliferative activity, after reaching 80–90% confluence, a "scratch" was applied with a sterile pipette tip to damage the cell monolayer. Then, 0.5 mg of lyophilized TEPs were placed into the experimental wells and cultivation was continued (n = 4). A day later, calcein AM dye solution (Lenreaktiv, Russia) at 1 : 3000 concentration in PBS (Biolot, Russia) was added to each well and incubated for 30 minutes in a CO_2 incubator. After washing twice with PBS, images were registered using a Zeiss LSM-880 microscope (Carl Zeiss, Germany) at a 494 nm wavelength and 517 nm registration. The relative residual defect area of the cell monolayer was determined.

Statistical processing of quantitative data was performed using nonparametric Mann–Whitney U test. Differences were considered significant at $p < 0.05$ (Statistics 7.0). Quantitative data were presented as median, 25% and 75% quartiles (Me, Q1; Q3).

RESULTS

Human umbilical cord-derived lyophilized tissue-engineered solid-state matrix (Fig. 1, a) is a ready-to-use porous drug formulation. The matrix does not contain cell nuclei of the original biomaterial, consists of collagen, retains glycosaminoglycans (GAGs) and essential basement membrane components. After decellularization of Wharton's jelly, collagen fibers retain their structural three-dimensional organization [7, 8]. Tissue-engineered hydrogel matrix is a product of matrix enzymatic treatment (by pepsin) and contains peptides, GAGs and free growth factors, previously fixed in tissue-engineered matrix structures. The hydrogel matrix has the ability to polymerize and gel under physiological conditions in vivo and in vitro. Tissue-engineered hydrogel matrix is an injectable dosage form that has shown efficacy in healing deep simulated synovial intra-articular cartilage defects [9]. However, lyophilized hydrogel matrix for the treatment of deep skin lesions seems to be the most convenient. Lyophilized hydrogel matrix can be used without the need for pre-processing. The dry form is very hygroscopic and actively absorbs wound exudate (unpublished data). If necessary, the product can be rehydrated into an injectable hydrogel matrix. Lyophilized tissue-engineered hydrogel matrix, similar to lyophilized tissue-engineered solid-state matrix, has a heterosporous structure (Fig. 1, b).

Implantation of both lyophilizate samples did not result in external signs of inflammatory response, such as edema, hyperemia, and temperature rise at the implantation site. As healing progressed, the wound areas gradually decreased. At week 3, the wound area in the control was 60.74% of the original defect area; the wound containing solid-state matrix lyophilizate was

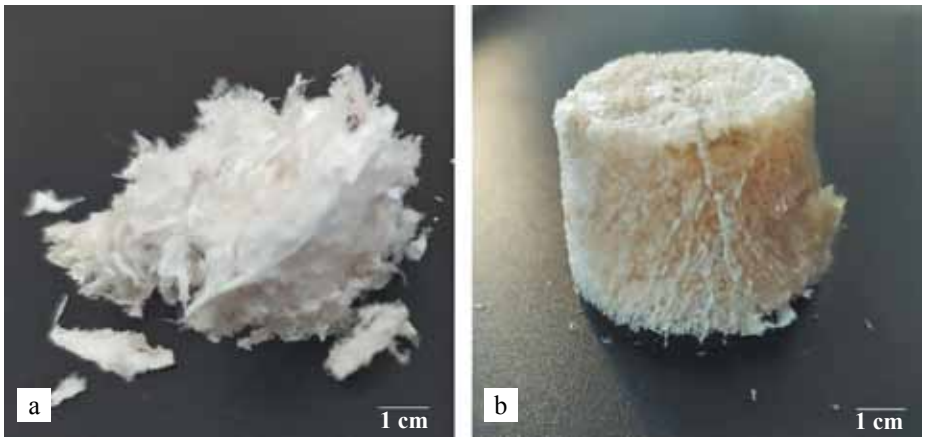

Fig. 1. Lyophilized tissue-engineered solid-state (a) and hydrogel (b) matrices

already 58.89% and the wound treated with hydrogel matrix lyophilizate was 55.02%. At week 4 and 5, planimetry results were as follows, respectively: 53.98% and 48.05% in the control; 51.08% and 30.34% in the presence of solid-state matrix lyophilizate; 50.27% and 42.69% when treated with hydrogel matrix lyophilizate.

Analysis of histological preparations at week 1 and 2 showed comparable values of the HAAI index in wounds with lyophilized TEPs and in the control. At day 7 after wound infliction, the HAAI index was 7.5 (7.0; 9.0) in the control, 7.0 (6.0; 8.0) in specimens containing tissue-engineered matrix and 7.0 (6.0; 8.0) in the tissue-engineered hydrogel matrix. The difference was not statistically significant. At week 2, the values were 6.0 (6.0; 6.0) with the control, 6.0 (6.0; 7.0) with the tissue-engineered matrix, and 6.0 (5.0; 7.0) with the hydrogel matrix. The difference was also not statistically significant. The results indicate that placing TEP (both solid and hydrogel forms) into the wounds did not cause an excessive inflammatory response, but at the same time did not inhibit the physiological process of inflammation.

Intense filling of the defect space with granulation tissue was observed in the experimental and control wounds already at week 1 (Fig. 2).

The density of the vascular bed in the tissues surrounding the wounds with lyophilized TEPs was statistically significantly higher than that in the control. The specific vascular area in the tissues surrounding the wounds at week 1 was 3.65% (3.65%; 3.77%), 5.63% (5.12%; 5.84%), and 4.76% (4.50%; 5.02%) in the control and experimental (lyophilized samples), respectively ($p = 0.009$ for solid-state matrix and $p = 0.028$ for hydrogel matrix compared to control). The mean diameter of vessels in the tissue surrounding the wound was 14.88 (13.92; 15.88), 10.40 (10.03; 10.64), and 10.88 (9.30; 11.57) μm in control and experimental samples, respectively ($p = 0.008$; $p = 0.007$). Specific area of granulation tissue vessels was not statistically significantly different in the control and experimental samples. As the follow-up period increased, the changes in these indicators were not statistically significant.

The most notable differences were observed in the formation of the epithelium when lyophilized TEPs were used. At week 1, epithelialization of the control wound (in contrast to the experiments) did not occur completely, with the thickness of the forming epithelium being 47.45 (46.65; 48.65) in the control wound, 73.76 (71.73; 75.77) in the wound containing solid-state matrix lyophilisate, and 75.35 (70.55; 79.83) μm in the wound with hydrogel matrix lyophilisate ($p = 0.0001$ for both lyophilisates as compared to the control). At week 2: 59.83 (58.22; 61.54), 91.90 (88.27; 92.02), 88.15 (85.73; 90.01) μm, respectively. At week 3: 68.98 (68.89; 71.74), 116.85 (110.74; 119.03), and 109.47 (106.12; 115.47) μm, respectively. At week 4: 88.83 (79.06; 90.84), 140.38 (133.47; 143.43), 132.56 (131.84; 138.45) μm, respectively. At week 5: 124.40 (121.64; 125.38), 159.16 (157.48; 161.99), 155.48 (152.12; 159.36) μm, respectively. The difference in these values in the experimental wounds, compared with the control, at all stages of the study, was statistically significant ($p = 0.0001$).

The forming epithelium of control wounds at week 1 consisted of irregularly shaped cells arranged in a thin layer. With the increase of the study period (3–4 weeks), the basal and spiny layers of the epithelium were clearly distinguished (Fig. 3). Epithelium formation was faster in the experimental wounds (Fig. 4). Clearly visible cell layers with a large number of Rete pegs were observed in the wounds containing hydrogel matrix already in the first week. Keratinocytes of the spiny layer in the wounds containing solid matrix were visualized larger and more mature already at week 2, they had large, rounded nuclei and were easily distinguishable from the cells of the basal and granular layers.

By the end of the study, the morphology of the cell layers of the epithelium of all wounds was indistinguishable from normal porcine skin (Fig. 4). When stained

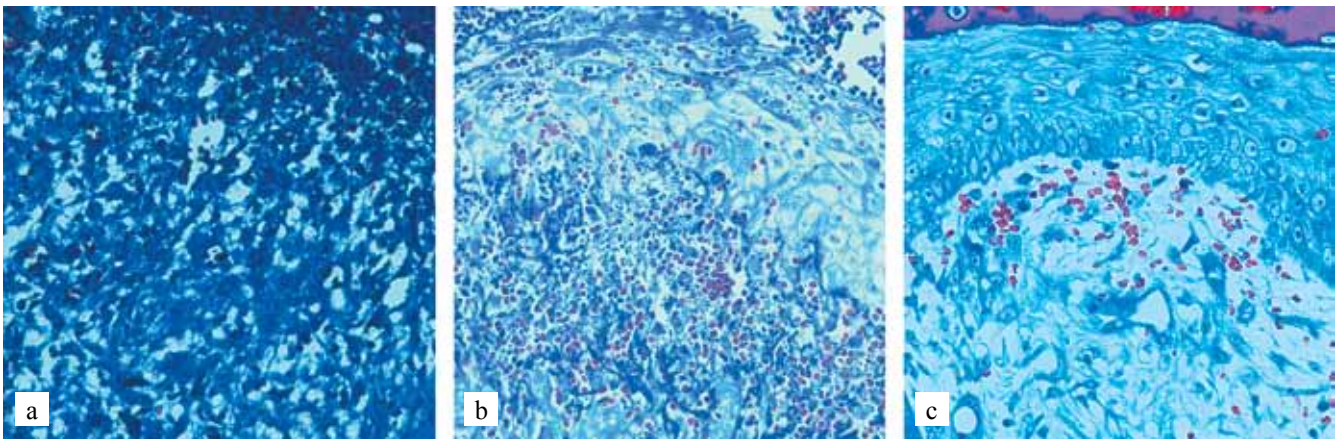

Fig. 2. Full-thickness skin wounds at week 1 in the control (a), wounds containing lyophilisates of tissue-engineered solid matrix (b) and tissue-engineered hydrogel matrix (c). Heidenhain's stain; 400×

with trichrome in samples from wounds containing lyophilized TEPs, formation of collagen structures in the granulation tissue area was observed as early as week 3 of the study. In the control, a similar pattern was observed only at week 5.

The solid-state matrix hydrolysate implanted into the wound bed was visualized in the granulation tissue at the initial stages of the study (Fig. 5) and gradually underwent biodegradation, moving through the papillary and reticular zones into the hypodermis.

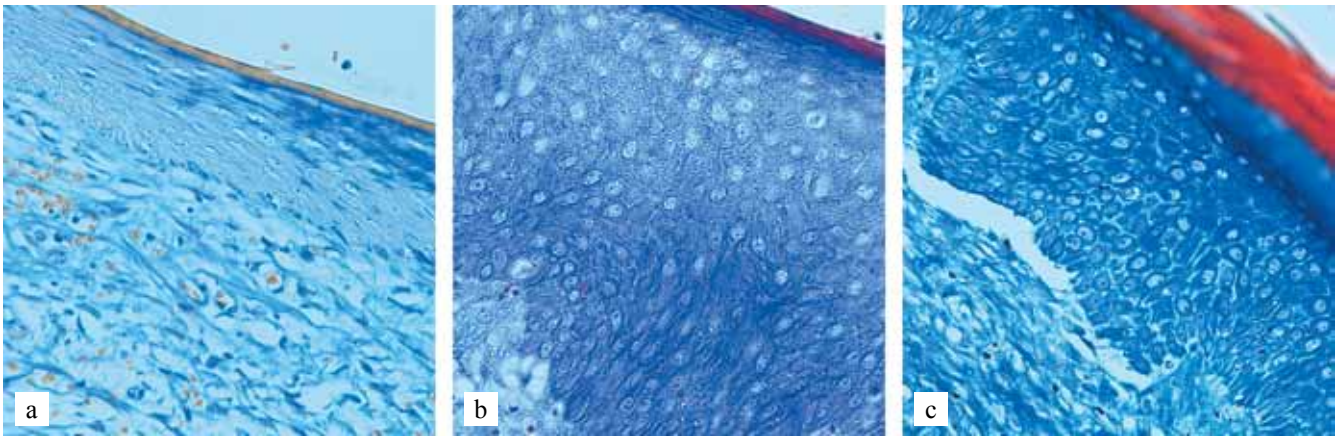

Fig. 3. Neoepithelium in the area of full-thickness wounds at week 3 in controls (a), wounds containing lyophilisates of tissue-engineered solid-state (b) and hydrogel matrices (c). Heidenhain's stain; 100×

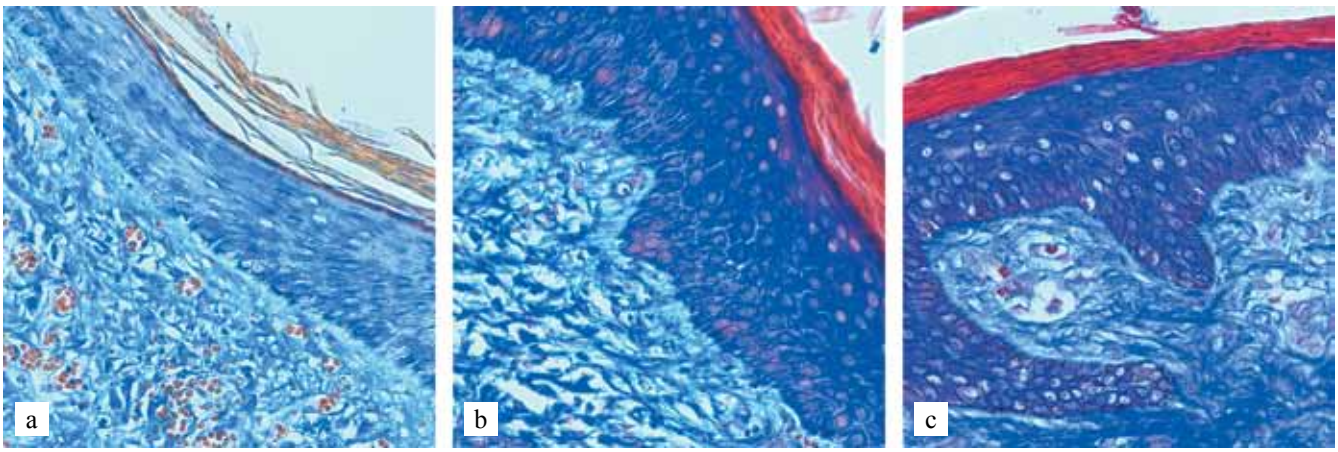

Fig. 4. Differentiated epithelium in the area of full-thickness wounds at week 5 in the control (a), wounds containing tissue-engineered matrix (b) and tissue-engineered hydrogel matrix lyophilisate (c). Heidenhain's stain; 400×

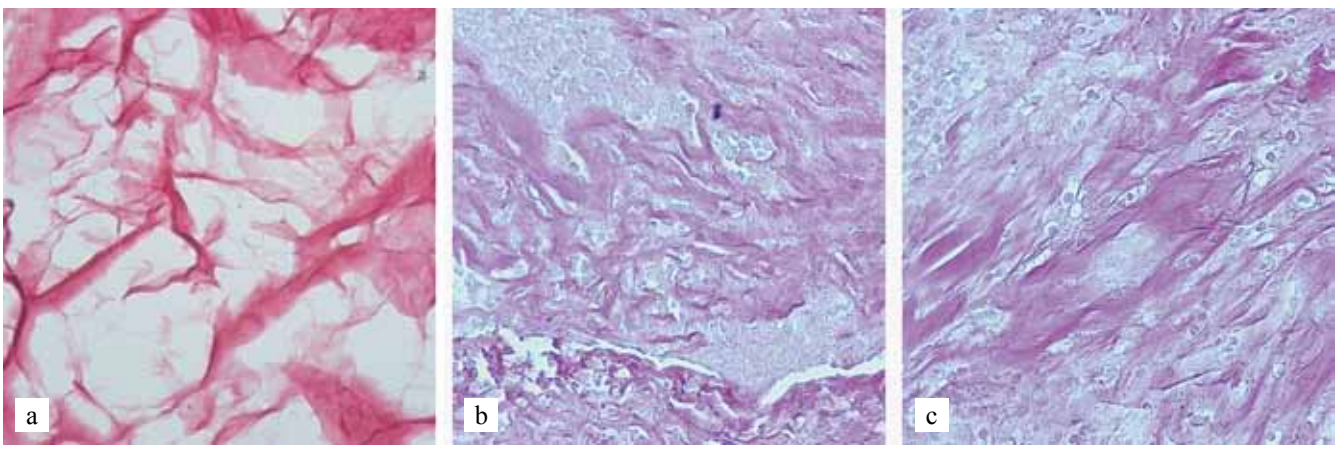

Fig. 5. Tissue-engineered solid-state matrix lyophilisate (a), lyophilisate with blood components at week 1 (b) and integrated lyophilisate of matrix in the hypodermis at week 5. Van Gieson's stain; 400×

The lyophilized hydrogel matrix implanted into the wound bed also underwent degradation and its traces gradually receded into the hypodermis by week 5 of the study (Fig. 6).

Importantly, no dense connective tissue capsule was formed around the degraded lyophilized TEPs and no massive infiltration with leukocytic cells was detected throughout the study period. This may imply that the products were not perceived as foreign by the recipient cells.

An in vitro study performed on cells isolated from mini pig full-thickness skin explants showed the absence of cytotoxic properties of soluble components of lyophilized TEPs. The metabolic activity of the cells in the Alamar Blue assay in the control was taken as 100%, while it was 101.66% (94.54%; 104.72%) and 100.76% (88.82%; 110.69%) for the solid-state and hydrogel matrices, respectively ($p = 0.916$).

The migratory activity of fibroblast-like cells isolated from full-thickness porcine skin explants and cultured in the presence of lyophilized TEPs was statistically significantly greater than that of the control. In the control, at day 1, the applied "scratch" was covered by 68.74% (63.74%; 71.26%) cells, while in the presence of lyophilized solid-state and hydrogel matrices, 75.43% (74.22%; 78.26%), and 76.74% (68.31%; 76.03%), respectively, with a statistically insignificant difference, compared to the control.

DISCUSSION

Skin wound healing is a sequential change in the phases of alteration, hemostasis, vascular responses and proliferation. Disruptions in this well-coordinated process can cause pathological healing. For example, the protective function of neutrophils (phagocytosis), necessary for wound cleansing against microorganisms and necrotic tissues, when in excess, leads to secondary alteration of surrounding tissues by active oxygen radicals, while differentiation of macrophages migrating to the wound defect area determines the outcome of proliferation and healing [10]. High-quality epithelialization is a guarantor of restoration of damaged skin function. These processes are based on numerous biochemical mechanisms and signaling pathways in which ECM plays a leading function [1]. Lyophilized products derived from human umbilical cord ECM that were placed into wounds, partially replenished the lost volume and served as a substrate for cell attachment. This mechanical function of lyophilized TEPs contributed to the rapid stopping of bleeding and filling of the wound bed with granulation tissue as soon as possible.

The results of the use of TEPs from ECM of different origin described in the literature showed that degradation rate, immunogenicity and ability to induce leukocyte infiltration are important predictors of biomaterial compatibility and efficacy [11]. The absence of local acute inflammatory response to the foreign body (lyophilisates of matrix and hydrogel matrix from the human umbilical cord for the pig recipient) and the absence of cytotoxicity of the obtained products for the mini-pig cells in vitro also contributed to the regenerative course of the healing process. Our previously published results indicating the non-cytotoxicity of TEPs for human dermal fibroblasts and for organ cells of different species of laboratory animals, suggest that cell-free products derived from human umbilical cord are biocompatible [8].

The healing outcome largely depends on restoration of the local vascular network delivering nutrients and removing metabolites. A significant advantage of human umbilical cord-derived lyophilized TEPs is the release of a large number of bioactive molecules (growth factors) during its biodegradation, which promote endotheliocyte migration and capillarogenesis. Structural reorganization of Wharton's jelly ECM during decellularization allows growth factors (such as vascular endothelial growth factor (VEGF), transforming growth factor (TGF-β), etc.) that are fixed to scaffold proteins to be present in lyophilized TEPs in higher concentrations compared to the original umbilical cord biomaterial [12, 13]. This study

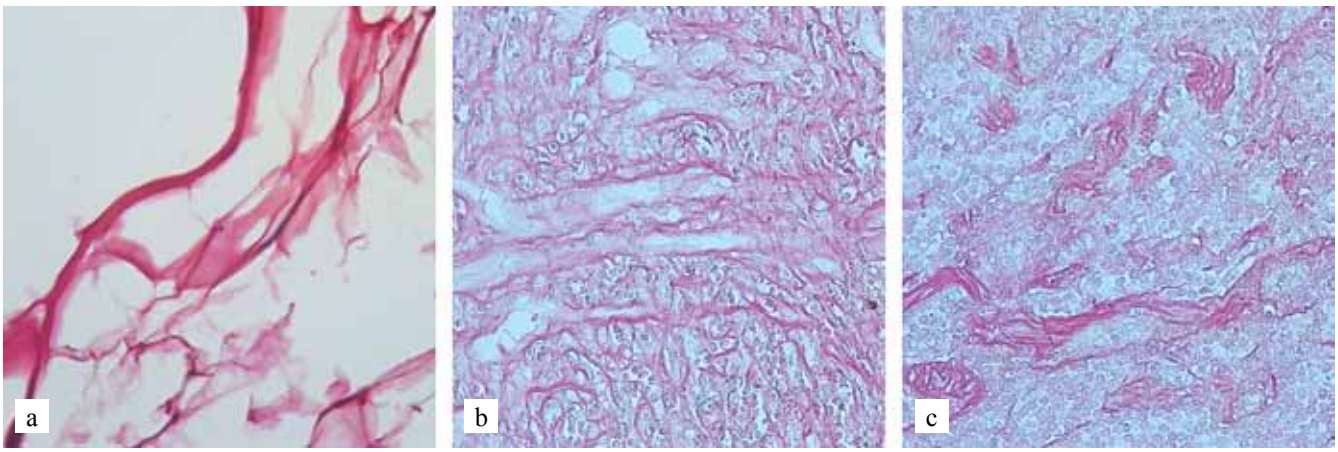

Fig. 6. Lyopholysate of hydrogel matrix (a), hydrogel matrix with blood components at week 1 (b) and integrated hydrogel matrix in the hypodermis at week 5. Van Gieson's stain; 400×

showed an increase in the specific area of the vascular bed in the tissues surrounding the wound with lyophilized TEPs and a simultaneous decrease in their diameter during the phase of fading of vascular responses, which may be a consequence of active formation of new capillaries. A similar phenomenon has been described in the scientific literature [14]. The greater in vitro migratory activity of pig skin cells in the presence of lyophilized TEPs, which we have established, confirms the assumption that lyophilized TEPs have chemotactic properties.

The main granulation tissue substance is deposited in the wound bed by fibroblasts. The origin of fibroblasts that form granulation tissue in deep full-thickness skin injuries has not yet been definitively elucidated. Studies in rabbits have identified vascular adventitia and pericytes below the panniculus carnosus and adipose layer as a source of granulation fibroblasts. In mice, it has been shown that circulating hematopoietic cells with mesenchymal characteristics can be transformed in situ in the wound. It is likely that multiple fibroblast subpopulations together form granulation tissue. In turn, the term "fibroblasts" refers to a very heterogeneous population of cells with different abilities to deposit ECM, differentiate into myofibroblasts and/or contract the wound. Fibroblasts in different skin areas can have different origins, and no single surface marker is intrinsic to all fibroblast lineages. This makes identification of the origin of fibroblasts in granulation tissue an interesting and challenging task [15].

Re-epithelialization of full-thickness wounds occurs exclusively from the wound edges. The suprabasal keratinocyte of the edge of the injured epidermis is elongated along the basal layer cell located under it and reaches the area of the wound bed, acquiring the ability to divide. Closure of the wound surface occurs as the next keratinocyte of the suprabasal layer of the wound edge undergoes the same process. Basal keratinocytes of the advancing epidermal layer have increased mitotic activity, have fewer desmosomes and more gap junctions. When the basement membrane is disrupted, human keratinocytes migrate along the temporal matrix, and it has been shown that their emerging cytoplasmic outgrowths are surrounded by laminin. But it remains unclear whether laminin serves as an adhesion substrate or is produced by cells in response to contact with collagen. Some studies have shown that migrating human keratinocytes do not produce basement membrane components [15].

According to the generally accepted notion, fibroblasts and endotheliocytes of the dermis grow into the primary clot that fills the wound space, while the epidermis grows on top, covering the granulation tissue [10]. There is an assumption that fibroblasts and pericytes are able to migrate from subcutaneous layers, hair follicles, and sweat glands, rather than from the relatively nonvascular and cell-populated reticular dermis [16]. In our study, mechanical filling of the defect area and gradual integration and revascularization of the graft, with formation of the recipient's native ECM, contributed to faster formation of mature epidermis.

Not only the mechanical filling of the wound, but also the qualitative composition of TEP, including structural elements of the basement membrane and functional molecules, play a role in the formation of quality mature epithelium. Statistically significant epithelial thickness was greater in wounds with lyophilized TEP compared to controls. Presumably, this may be a consequence of both the presence of growth factors in them and the basement membrane molecules such as type IV collagen and laminin that we identified in lyophilized TEPs [7]. In their in vitro studies, Deshpande et al. observed that inclusion of fibroblasts into the cell-free dermal matrix lacking basement membrane components had no significant effect on epithelium formation [14].

In wounds with tissue-engineered hydrogel matrix lyophilizate injected, differentiation and maturation of epitheliocytes were most active, not only as compared to the control, but also as compared to tissue-engineered solid-state matrix lyophilizate. At week 1, they showed signs of formation of Rete epidermal thickenings, while in the control wound, even the formation of a thin layer of epitheliocytes was incomplete at this time of the study. The distinct differentiation of epithelial cells, characterizing their maturity, was more noticeable at week 2 of the experiment when hydrogel matrix was used.

CONCLUSION

Our experimental studies on the effect of cell-free lyophilisates of tissue-engineered matrices on isolated cellular material in vitro and healing in an in vivo model showed that these lyophilisates were biocompatible and bioactive. They promote a regenerative type of deep skin wound healing when implanted in vivo more rapidly than in the control, forming a mature epithelial layer. The lyophilized hydrogel form of the tissue-engineered matrix promotes epidermal maturation with the formation of epidermal thickening faster than the lyophilized solid-state matrix. Presumably, this effect can be due to the action of growth factors present in the hydrogel form of the matrix in the unbound state. The results obtained make it possible to develop a scientifically substantiated program of preclinical studies on the safety and efficacy of human umbilical cord-derived lyophilized TEPs in accordance with GOST R 56699-2015 requirements.

The authors declare no conflict of interest.

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