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PEDIATRIC ORGAN TRANSPLANTATION IN THE RUSSIAN FEDERATION

Глубокоуважаемые коллеги!

Перед вами первый выпуск журнала за 2020 г. Одной из важнейших составляющих профессионального информационного поля и значимой частью содержания «Вестника…» являются ежегодные сообщения Регистра Российского трансплантологического общества, которые мы публикуем вот уже более 10 лет, обычно во втором номере, и которые позволяют оценить состояние клинической трансплантологии в стране в целом, в отдельных регионах и трансплантационных центрах, а также из года в год наблюдать динамику развития этой непростой отрасли здравоохранения.

Предваряя второй выпуск журнала с очередным сообщением Регистра, хотелось бы анонсировать те его аспекты, которые связаны с трансплантацией органов детям.

Отмечается очевидная положительная динамика в оказании трансплантационной помощи детям. За 2019 год в РФ было выполнено 2427 пересадок органов (16,5 на 1 млн населения), из них более 200 – детям. При распределении донорских органов дети, состоящие в листах ожидания, имеют приоритет.

Полностью решена проблема трансплантации печени детям. Операции выполняются всем выявленным и направленным в трансплантационные центры реципиентам. Полностью прекращена практика выезда за рубеж для детской трансплантации печени.

Отдельным контингентом педиатрических пациентов являются дети, которым выполняется одномоментная трансплантация печени и почки. Эти операции выполняются только в НМИЦ ТИО им. Шумакова, в том числе оперированы дети с массой тела 8–9 кг. Нашим

Dear colleagues!

Here is the first issue of our journal for 2020. Annual reports from the Registry of the Russian Transplant Society are one of the most important components and a significant part of the Russian Journal of Transplantology and Artificial Organs. We have been publishing these reports for over 10 years – usually in the second issue. The reports give us the opportunity to evaluate the state of clinical transplantology in the country as a whole, in certain regions and transplant centers. They also enable us to, from year to year, observe the trend in the development of this complex sector of the healthcare industry.

As we await the second issue of the journal with the next report from the Registry, I would like to announce those aspects that are related to pediatric organ transplantation.

Provision of pediatric transplant care has an obvious positive trend. In 2019, about 2427 organ transplantations were performed in the Russian Federation (16.5 per million population) of which more than 200 were pediatric transplants. Waitlisted children are given priority in the distribution of donor organs.

The problem of pediatric liver transplantation has been completely addressed. All recipients identified and referred to transplant centers undergo surgical interventions. The practice of traveling abroad for pediatric liver transplantation is now a thing of the past – it has completely stopped.

Children undergoing simultaneous liver-kidney transplantation are a separate contingent of pediatric patients. These operations are carried out only at Shumakov National Medical Research Center of Transplantology and Artificial Organs.

мировым приоритетом является разработанная и опубликованная в American Journal of Transplantation методика одновременного лапароскопического изъятия фрагмента печени и почки у живого донора.

Опыт выполнения трансплантаций почки детям имеется в 16 центрах трансплантации (Москва, Санкт-Петербург, Новосибирск, Кемерово, Казань, Уфа, Оренбург, Саратов, Омск и Воронеж). Готовится к началу работы в области педиатрической трансплантации почки Санкт-Петербургский государственный педиатрический медицинский университет, начала работу программа в НИИ детской неотложной хирургии и травматологии в Москве.

Отдельным вопросом является трансплантация сердца детям. Дети с массой тела от 20 кг получают трансплантаты от взрослых посмертных доноров небольших антропометрических параметров. Успешный опыт таких операций имеется в НМИЦ ТИО им. Шумакова, НМИЦ им. Алмазова, в Краснодаре и Новосибирске (всего 10–15 операций в год).

В то же время остается не решенной проблема пересадки сердца детям с массой тела менее 20 кг, что подразумевает необходимость начала работы в области посмертного донорства органов детей, которое предусмотрено статьей 47 ФЗ 323 и Инструкцией по констатации смерти мозга.

It includes children weighing 8–9 kg who underwent surgery. Our global priority is the technique for simultaneous laparoscopic removal of liver and kidney fragment from a living donor, which was developed and published by the American Journal of Transplantation.

About 16 transplant centers (Moscow, St. Petersburg, Novosibirsk, Kemerovo, Kazan, Ufa, Orenburg, Saratov, Omsk and Voronezh) have successfully performed pediatric kidney transplantation. The St. Petersburg State Pediatric Medical University is preparing to start activities on pediatric kidney transplantation; a program at the Research Institute of Pediatric Emergency Surgery and Traumatology in Moscow has kicked off.

Pediatric heart transplantation is a separate matter altogether. Children weighing 20 kg or more receive transplants from adult posthumous donors that have small anthropometric indicators. Such surgical interventions have been successfully performed at Shumakov National Medical Research Center of Transplantology and Artificial Organs, Almazov National Medical Research Centre, as well as in Krasnodar and Novosibirsk (a total of 10 to 15 operations per year).

However, the issue of heart transplantation for pediatric patients weighing less than 20 kg remains a major challenge. This implies the need to kick start work on posthumous organ donation from children. This issue is envisaged by article 47 of Federal Law 323 of the Russian Federation and by the Guidelines for the Determination of Brain Death.

С уважением
академик РАН С.В. Готье

Sincerely,
Sergey Gauthier, Academician,
Russian Academy of Sciences
Editor-in-Chief, The Russian Journal of Transplantology and Artificial Organs

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DIAGNOSTIC VALUE OF GALECTIN-3 IN HEART TRANSPLANT RECIPIENTS WITH MYOCARDIAL COMPLICATIONS

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Objective: to determine the diagnostic value of galectin-3 in transplant recipients with myocardial fibrosis and acute heart transplant rejection, verified by endomyocardial biopsy. **Materials and methods.** The study included 124 patients with end-stage heart failure. Their ages ranged from 16 to 71 (average 48 ± 12) years, of which 106 (85%) were men and 18 (15%) were women. From 2013 to 2016, these patients underwent a heart transplant procedure at the Shumakov National Medical Research Center of Transplantology and Artificial Organs, Moscow, Russian Federation. Analysis of endomyocardial biopsy specimens was used to verify acute cellular, humoral rejection and myocardial fibrosis of the heart transplant. Severity and nature of fibrosis was evaluated using a qualitative imaging technique. Galectin-3 concentration was measured by enzyme immunoassay using Human Galectin-3 Platinum ELISA reagent kits (Bender MedSystems GmbH, Vienna, Austria). **Results.** In the long-term post-transplantation period, in comparison with the early post-transplantation period, the number of verified graft myocardial fibrosis increased by 88% in recipients who had acute rejection crises and by 37% in recipients who had no rejection crises. Graft myocardial fibrosis was detected more often in recipients who had antibody-mediated rejection than in those who had acute cell rejection (92% vs 75% of cases, respectively). Plasma galectin-3 levels in recipients with graft myocardial fibrosis was higher than in recipients without it ($p = 0.05$ 1 year and $p = 0.01$ 1–5 years after heart transplantation). In recipients who had acute rejection crises, the risk of developing graft myocardial fibrosis was 1.64 (RR = 1.64 ± 0.1 [95% CI 1.1–2.2]). **Conclusion.** Galectin-3 is a biomarker for myocardial fibrosis in acute heart transplant rejection.

Keywords: heart transplantation, galectin-3, myocardial fibrosis, acute rejection, diagnostic value.

INTRODUCTION

The current achievements in heart transplantation (HT) have provided higher survival and improved quality of life for recipients. In the long term after transplantation, heart recipients have an increased risk of developing subclinical chronic heart failure, resulting from a combination of various pathological factors leading to the formation of transplant myocardial fibrosis, such as arterial hypertension, acute transplant rejection, transplant vasculopathy, and other diseases [1].

Endomyocardial biopsy (EMB) is an objective method for verification of myocardial pathology. After transplantation, EMB is performed within the time required by the treatment protocol or as indicated; however, there are some limitations and risks inherent in all invasive diagnostic methods. Moreover, when examining a biopsy sample, a myocardial fragment is evaluated which may not reflect the state of other areas that did not fall into the test material. The development of minimally invasive methods for identifying complications in the post-transplant period is performed, with the aim of improving preclinical diagnosis, considering, among others, the need to reduce the number of repeated invasive diagnostic interventions, partially replacing them with functional and/or laboratory tests which can not only detect the presence of transplant myocardial fibrosis, but also control the effectiveness of the recipient treatment.

Particular attention is paid to the identification of profibrogenic biological agents that can be indicators of the risk of negative cardiovascular events associated with the development of fibrosis [2, 3]. The relatively recently described biomarkers for the development of heart failure and myocardial fibrosis include galectin-3 which belongs to the lectin family and plays an important role in the regulation of myofibroblast proliferation, immune response, inflammation and remodeling of heart vessels [4, 5]. At the site of injury, galectin-3 is secreted into the extracellular space and promotes the develop-

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ment of fibrosis through the activation of fibroblasts [6]. In the role of heart recipients, the role of galectin-3 has been less studied, but it has been found that its level in blood plasma is higher in patients with graft myocardial fibrosis [7]. The aim of this study was to determine the diagnostic efficacy of galectin-3 in recipients with myocardial fibrosis and acute graft rejection, verified by endomyocardial biopsy.

MATERIALS AND METHODS

The study included 124 patients with heart failure of the III–IV functional class according to the classification of the New York Heart Association (NYHA) aged 16 to 71 (average 48 ± 12), 106 (85%) men and 18 (15%) women. In 2013 to 2016, at the Shumakov National Medical Research Center of Transplantology and Artificial Organs (Moscow, Russia), the patients have got heart transplants. In 67 recipients (51 men and 16 women, aged 16 to 71; 41 ± 12), heart failure was caused by dilatation cardiomyopathy, and 57 (55 men and 2 women, aged 37 to 70; 57 ± 8 years old) had coronary artery disease (CAD).

All patients indicated for HT underwent a routine examination in compliance with the protocol of patient management at the Shumakov National Medical Research Center of Transplantology and Artificial Organs and National Clinical Recommendations [8]. Routine examination of recipients included clinical examination, thermometry, virologic and bacteriological analyses, CBC and blood biochemistry in dynamics with determination of tacrolimus concentration, daily monitoring of blood pressure, echocardiography, myocardial biopsy, and annual coronary angiographic examination.

Acute cellular and humoral rejection, as well as transplant myocardial fibrosis, were verified on the basis of a study of endomyocardial biopsy samples. For histological examination, pieces of endomyocardium were fixed in 10% formalin, then washed with water, dehydrated, and embedded in paraffin. 3–4 microns thick slices were prepared on a microtome. To verify myocardial fibrosis of the transplant, slices were Masson Trichrome stained which made it possible to clearly distinguish between connective tissue, which, depending on its maturity, is stained in various shades of blue and differs from other myocardial tissues.

Fig. 1 shows examples of histological preparations of transplanted heart biopsy specimens where fibrotic changes of various types are detected: focal, diffuse, and diffuse focal fibrosis.

To diagnose the acute cellular rejection (ACR), the slices were hematoxylin and eosin stained; to diagnose the antibody-mediated rejection (AMR), immunohistochemical tests were used. The degree of acute cellular and humoral transplant rejection was evaluated according to the recommended classifications adopted by the International Society for Heart and Lung Transplantation (ISHLT-2004 and ISHLT-2013).

Venous blood plasma served as a material for studying the concentration of galectin-3. The concentration of galectin-3 was measured by enzyme immunoassay with the Human Galectin-3 Platinum ELISA reagent kits (Bender MedSystems GmbH, Vienna, Austria).

Sensitivity and specificity, the selection of the optimal cutoff threshold and of the best diagnostic strength test were determined by ROC analysis. To assess the diagnostic significance of galectin-3, a relative risk indicator was used.

The analysis of the obtained data was performed by the standard statistical processing methods with Microsoft Office Excel and the IBM SPSS STATISTICS 20 software package for scientific and technical calculations (IBM SPSS Inc., USA).

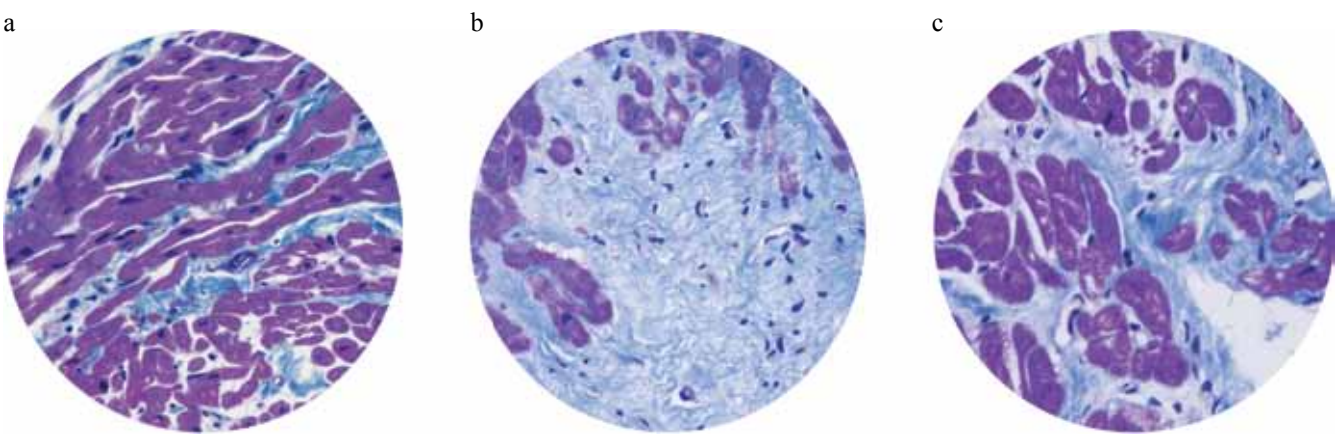

Fig. 1. Histological preparations of endomyocardial biopsy specimens. Coloring according to Masson ×400 (connective tissue is colored blue, cardiomyocytes are pink): a – diffuse growth of loose fibrous connective tissue with single cells of the fibroblastic line, focal protein granular dystrophy of cardiomyocytes; b – focal growth of unformed connective tissue with single cells of the connective tissue row, moderate protein dystrophy of cardiomyocytes; c – diffuse focal growth of loose fibrous connective tissue, in which proliferation of connective tissue cells is noted, focal protein dystrophy of cardiomyocytes

RESULTS AND DISCUSSION

The histological signs of transplant myocardial fibrosis at different times after HT were detected in 124 recipients selected by random sampling from a total of 432 recipients who underwent HT in 2013–2016 at the Shumakov National Medical Research Center of Transplantology and Artificial Organs. 583 endomyocardial biopsies (from one patient: from 3 to 20; on average 5 ± 2 EMB) obtained in the following periods after transplantation: early period – the first month after HT (30 ± 14 days), a year later (334 ± 69 days) and after 1–5 years (963 ± 273 days) were studied.

By the end of the first month after HT, 58 (46%) of the heart recipients included in the study showed transplant myocardial fibrosis; by EMB, 66 (54%) recipients showed no histological signs of fibrosis. One year after HT, 74 (60%) recipients, and 1–5 years later, 95 (77%) recipients developed verified transplant myocardial fibrosis (Fig. 2).

The presented data reflect the rate of detecting fibrotic changes in the graft myocardium in the randomly selected recipients of the studied group and cannot be extrapolated to the entire population of heart recipients operated at the Shumakov National Medical Research Center of Transplantology and Artificial Organs in 2013–2016. At the same time, the results allow us to state, firstly, a fairly frequent, in almost half of cases, detection of transplant myocardial fibrosis even in the early period after HT; secondly, we can note the significant increase in the proportion of cases of transplant myocardial fibrosis in recipients with the time after HT: in the present study, by 67% after 1–5 years compared with the early period.

Obviously, in the early post-transplant period, the cause of myocardial fibrosis may be the presence of fibrotic changes in the myocardium of the donor heart. It should be noted that the age of the heart donors for the recipients included in this study was 42 ± 11 (18 to 64) years. Studies of the donor heart to detect fibrotic changes have not been performed; however, the implementation of such an analysis will be appropriate to study the factors affecting the development of transplant myocardial fibrosis and the long-term prognosis of heart recipients.

The analysis of the rate of detection of various types of fibrosis in the early and long-term periods after HT showed that by the end of the 1st month after transplantation of 58 recipients with transplant myocardial fibrosis, diffuse fibrosis was verified in 13 (22%), focal fibrosis in 40 (69%) and 5 (9%) – diffuse focal fibrosis. After 1–5 years after HT, diffuse fibrosis was verified in 20 (21%) of 95 recipients with myocardial fibrosis, focal in 55 (58%) and diffuse-focal fibrosis in 20 (21%). Diffuse fibrosis develops in the interstitial or perivascular space, it is accompanied by excessive deposition of type I collagen in the myocardium due to the predominance of its synthesis over decay [9]. With the development of focal fibrosis, dead cardiomyocytes are replaced by connective tissue and are accompanied by excessive deposition of type III collagen [10]. According to the results of this study, there is an increase in the proportion of the most severe, diffuse focal forms of fibrosis in the study group of recipients: from 9% in the early post-transplant period to 21% in the long term.

Earlier, it has been previously found that the level of galectin-3 in blood plasma is higher in recipients with graft myocardial fibrosis; higher values were found in diffuse focal fibrosis [7]. To determine the diagnostic significance of galectin-3, the ROC curve analysis was used as a marker of transplant myocardial fibrosis (Fig. 3).

Calculations showed that the area under the ROC curve of galectin-3 1–5 years after transplantation in recipients with transplant myocardial fibrosis was 0.765 ± 0.060 [0.64–0.88], p = 0.00.

The threshold levels of galectin-3 significant for the diagnosis of transplant myocardial fibrosis 1–5 years after heart transplantation, were determined at a point by the optimal combination of sensitivity and specificity. In the diagram of the dependences of sensitivity and specificity on the concentration of galectin-3 in blood plasma, the intersection point of the curves reflects the threshold level (Fig. 4).

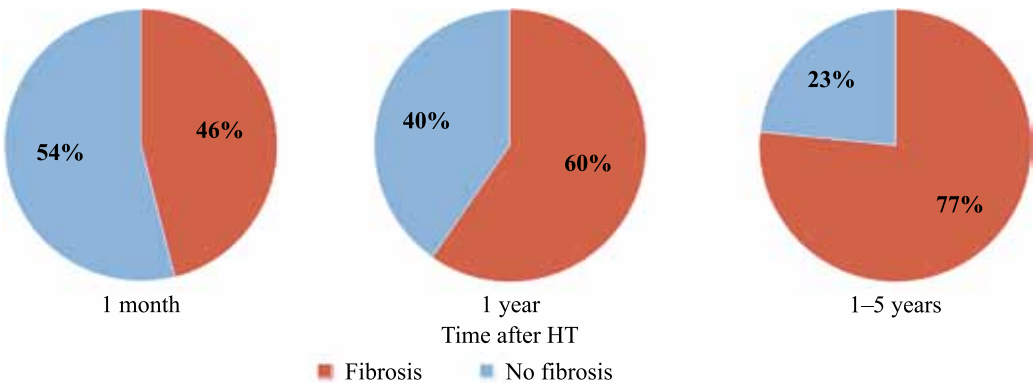

Fig. 2. Percentage of verified myocardial fibrosis in the recipients at different times after HT

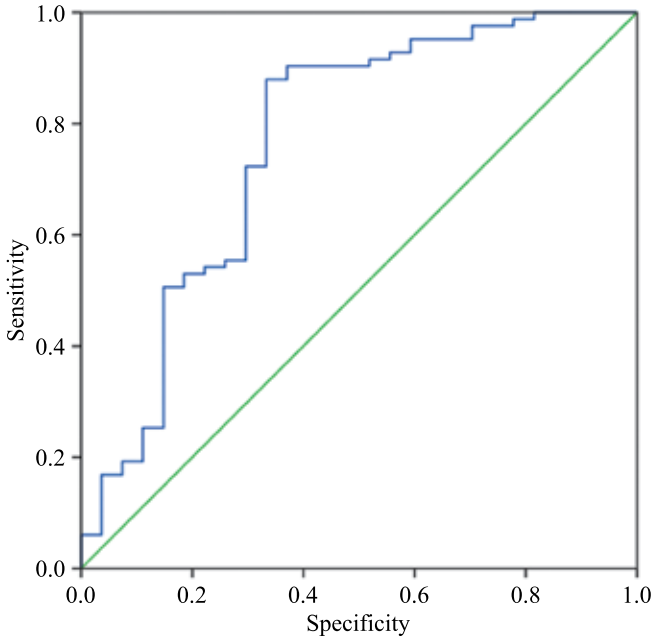

Fig. 3. Galectin-3 ROC curve 1–5 years after HT in recipients with transplant myocardial fibrosis

The threshold value of galectin-3 significant for the diagnosis of transplant myocardial fibrosis in the long term after HT, was 16.9 ng/ml. At a galectin-3 level exceeding the found threshold value, the probability of transplant myocardial fibrosis risk in heart recipients is 1.6 times higher (RR = 1.6 ± 0.1 [95% CI 1.2–2.0]), than in recipients with a galectin-3 level below this threshold value (sensitivity – 71%, specificity – 70%).

Among the main factors limiting the survival of heart recipients in the early postoperative period and during the first year of life after HT is acute rejection of the transplanted heart. The reaction of rejection of a heart transplant is a manifestation of the protective reaction of the recipient's organism against foreign cells of the donor organ, includes mechanisms of an innate, cellular and antibody-mediated (humoral) immune response, as a result of which episodes of acute transplant rejection are a factor stimulating the development of fibrotic changes in the transplanted heart.

Among the 124 patients included in this study, 75 (60%) recipients suffered episodes of acute rejection of the transplanted heart in the early post-transplant period. A year after transplantation, already 89 (72%), and after 1–5 years – 92 (74%) of heart recipients suffered acute rejection episodes.

Of the patients who suffered acute transplant rejection episodes, myocardial fibrosis in the early stages after HT was detected in 31 (41%) of 75 recipients; a year later, in 51 (57%) of 89 recipients; after 1–5 years, it was verified in 71 (77%) of 92 recipients.

In patients without acute rejection episodes, graft myocardial fibrosis was detected in 27 (55%) of 49 heart recipients in the early stages; a year later, myocardial fibrosis was verified in 23 (65%) of 35 recipients; after 1–5 years – in 24 (75%) of 32 recipients.

Although in the long term after transplantation, the proportion of recipients with verified myocardial fibrosis practically did not differ in the groups who underwent and did not undergo acute rejection (77% and 75%, respectively), the analysis showed that in recipients of the heart who underwent acute rejection episodes, the proportion of recipients with myocardial fibrosis after 1–5 years compared with the early period after transplantation increased by 88%, and in recipients who did not suffer acute rejection episodes – by 37% (Fig. 5).

The relationship between the episodes of antibody-mediated rejection and the development of transplant myocardial fibrosis is more pronounced: in recipients of the heart who underwent humoral rejection, transplant myocardial fibrosis is detected in long-term periods in

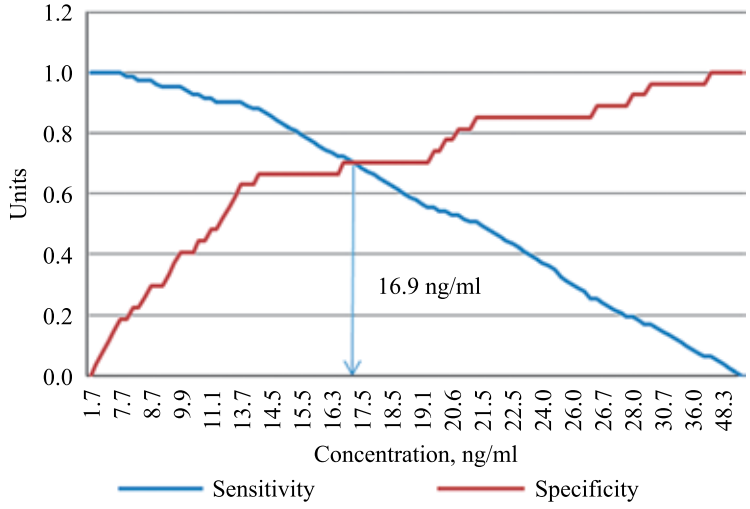

Fig. 4. The diagnostic significant threshold value of the galectin-3 1–5 years after HTx for transplant myocardial fibrosis

92% of cases, and in recipients who have experienced acute cell rejection episodes – in 75% of cases (Fig. 6).

The detected differences seem to be associated with an additional negative effect of immune factors acting upon humoral rejection of the graft myocardium.

The results of the present study confirm the idea of the effect of episodes of acute cellular and antibody-mediated rejection on the development of fibrotic changes in a transplanted heart. Obviously, the formation of the latter is promoted by edema developing during acute rejection, macrophage and lymphocyte infiltration, production of activated inflammatory cells and fibroblasts of pro-inflammatory and profibrogenic mediators, etc. [11, 12].

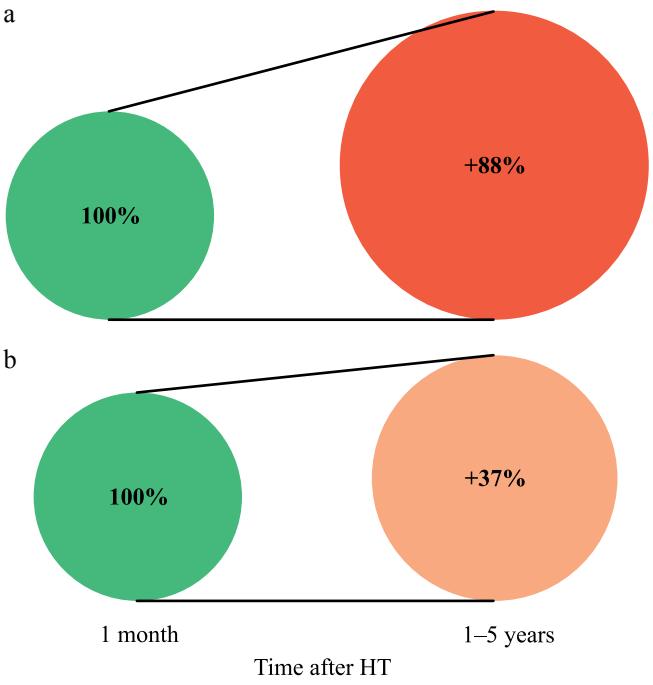

Fig. 5. An increase in the number of verified cases of graft myocardial fibrosis in recipients with (a) and without (b) episodes of acute rejection

A comparative analysis of the diagnostic efficacy of galectin-3 in myocardial fibrosis in recipients who underwent and did not undergo acute transplant rejection showed the following. In the long term after HT, the concentration of galectin-3 in recipients who underwent and did not undergo acute transplant rejection was 20.57 [13.92; 27.24] and 15.25 [12.06; 19.47] ng/ml, respectively, p = 0.00. In patients with transplanted heart myocardial fibrosis and without fibrosis, according to EMB, the level of galectin-3 was 20.60 [14.52; 26.29] and 15.36 [11.95; 22.42] ng/ml, respectively, p = 0.05 [7].

At galectin-3 concentration exceeding the calculated threshold value (16.9 ng/ml), the relative risk of myocardial fibrosis in the long term after transplantation in recipients who underwent acute rejection episodes was RR = 1.64 ± 0.1 [95% CI 1.1–2.2] (sensitivity – 71%, specificity – 75%).

In recipients with galectin-3 level above 16.9 ng/ml but not suffering acute rejection, the relative risk of developing myocardial fibrosis was RR = 1.38 ± 0.2 [95% CI 0.8–2.3] (sensitivity – 71%, specificity – 57%) and was not statistically significant (the boundaries of the confidence interval included one).

Thus, in the long term after HT, galectin-3 concentration in the blood plasma has diagnostic value in rela-

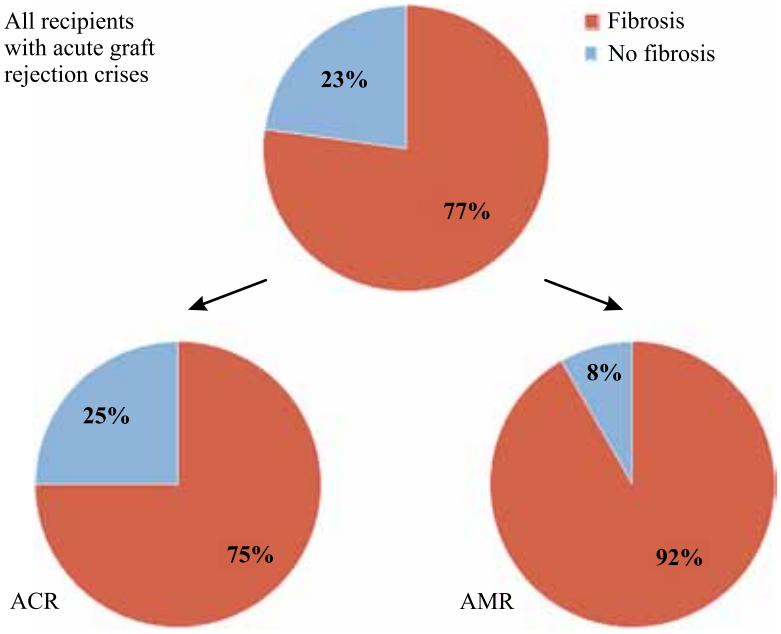

Fig. 6. The frequency of detection of myocardial fibrosis (%) after 1–5 years after HT in recipients with acute cellular (ACR) and humoral rejections (AMR) graft episodes

tion to transplant myocardial fibrosis in recipients who underwent acute rejection crises. At the same time, in recipients who did not undergo rejection crises, the test for galectin-3 to detect transplant myocardial fibrosis is insignificant. Most likely, the latter is associated with a lesser activity of the processes of fibrogenesis that occur in the body of recipients who have not undergone acute rejection, which is also indicated by differences in the increase in the number of verified cases of graft myocardial fibrosis in recipients who underwent (by 88%) and who did not (37%) crises of acute rejection, for a period of 1 month to 1–5 years after HT. In recipients of the heart who have suffered crises of acute transplant rejection, at galectin-3 ≥16.9 ng/ml, the risk of developing myocardial fibrosis is 1.64 times higher than in recipients with a galectin-3 level below the threshold.

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The authors declare no conflict of interest.

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ISOLATED NON-COMPACTION OF THE LEFT VENTRICULAR MYOCARDIUM: A CLINICAL AND MORPHOLOGICAL STUDY

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Isolated left ventricular noncompaction (LVNC) in adult patients is a rare form of primary cardiomyopathy. There have been several morphological studies of this condition in which a heart transplantation was performed. **Objective:** to analyze literature and clinical cases of patients with LVNC, macroscopic and histological data of the removed hearts. **Materials and methods.** At our center three patients (2 women aged 18 and 31, and 1 man aged 49) were morphologically diagnosed with LVNC had a heart transplant. We retrospectively analyzed the clinical, macroscopic and histological data of the removed hearts in these patients and the results of the transplants performed. Light microscopy of a fixed myocardial preparation stained with hematoxylin and eosin was used for histological examination. **Results.** A histological examination confirmed the presence of LVMI in these patients. **Conclusion.** LVMI is a rare disease, that can occur asymptomatic or cause severe congestive heart failure, requiring transplantation.

Keywords: isolated left ventricular noncompaction, heart transplantation.

Isolated left ventricular noncompaction (LVNC) is a rare non-compaction cardiomyopathy. More often than not, left ventricular noncompaction (LVNC) is combined with other heart defects. There are known observations of biventricular noncompaction. All forms of non-compact myocardium are rare observations, characterized by abnormal myocardial embryogenesis, resulting in preservation of inter-trabecular sinusoids and development of spongy myocardium [1]. Normally, the process of ventricular myocardial compaction, which occurs within 5–8 weeks of fetal development, begins from the epicardium, spreading to the endocardium and from the basal sections to the apex of the heart [2]. With isolated LVNC, the left ventricular wall, especially in the apex region, becomes thicker. However, this occurs due to increased thickness of the spongy layer, while the compact layer, on the contrary, remains thin [3]. Isolated LVNC is diagnosed via echocardiography and/or magnetic resonance imaging, contrast ventriculography is rarely used [4]. Endomyocardial biopsy in isolated LVNC [5], in our opinion, is uninformative and inappropriate, because fragments of the subendocardial zone fall into the biopsy.

Differential diagnosis of isolated LVNC should be performed with idiopathic dilated cardiomyopathy [6–8] and with corrected transposition of the great vessels, where the right ventricle (RV) is located in the position of the left ventricle (LV), which can be mistaken for a non-compact myocardium. Clinical symptoms depend on the volume and location of a non-compact myocardium. Often the disease is manifested by ventricular arrhythmia, which requires implantation of a cardioverter defibrillator. These patients are often mistakenly diagnosed with idiopathic dilated cardiomyopathy [9].

In symptomatic patients with isolated LVNC, a developing heart failure is predominantly associated with systolic and diastolic dysfunction of the LV. Systolic dysfunction arises from significant reduction in the compact layer, and not as a result of relative myocardial ischemia due to a mismatch between myocardial need and the amount of oxygen delivered, as suggested by Y. Agmon et al. [3]. Diastolic dysfunction mechanism includes a combination of pathological relaxation and deficiency of blood supply to the LV as a result of hypertrabeculation [3]. Heart failure in isolated LVNC can occur at any age, from infants to the elderly.

According to M. Greutmann et al. [10], of the total of 115 patients, 77% had symptoms of the disease. Compared to the asymptomatic patients, the symptomatic patients were significantly older and had larger left ventricular cavities and worse left ventricular ejection fraction (EF). During a median follow-up of 2.7 years (range 0.1 to 19.4), none of the asymptomatic patients died or underwent heart transplantation, compared to 31% (27 of 88) of the symptomatic patients ($p = 0.001$). In patients with NYHA class III or above, cardiovascular complications are the main predictors of adverse outcomes. Left ventricular dilatation and systolic dysfunction are less significant predictors [10].

LVNC is more often combined with congenital heart defects [11], but there is also an isolated form, which can equally cause severe heart failure [12]. These patients have unfavorable outcomes due to accelerated develop-

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ment of fatal complications – arrhythmias, thromboembolism and severe left ventricular decompensation of blood circulation [13]. Therefore, heart transplantation is the only and radical treatment option for isolated LVNC patients who develop severe congestive heart failure.

T. Spieker et al. [14] presents the results of echocardiography and pathomorphological examination of the hearts of two newborn boys with isolated LVNC, who underwent heart transplantation at 40 days of age. According to the authors, it is very difficult diagnosing isolated LVNC in newborns because compared to adult patients, pathological changes in the myocardium are much less pronounced.

W.A. Zuckerman et al. [15] performed a retrospective analysis of mortality and heart transplant outcomes in pediatric patients with isolated LVNC who were treated at Morgan Stanley Children's Hospital in New York from January 1993 to September 2009. LVNC was diagnosed in 50 patients, 34 of them were less than 1 year of age. Twenty-six patients died or underwent a heart transplant. Patients surviving 1 year after presentation had 75% conditional survival, and patients surviving 2 years after presentation had 92% conditional survival. Independent predictors of poor outcome were hemodynamic instability, decreased ventricular function, and left ventricular dilatation. Of the 21 patients who presented with hemodynamic instability, 17 died or underwent transplantation at a median of 0.08 years after diagnosis with isolated LVNC. The authors conclude that heart transplantation is necessary for children with isolated LVNC as early as possible after diagnosis of the disease.

Until 2001, only six patients with isolated LVNC underwent heart transplantation [4]. These authors describe diagnostic difficulties in examining a young woman with neurological symptoms, atrial fibrillation, and severe systolic dysfunction, which ultimately led to heart transplant. This was the seventh surgery in a similar group of patients.

S. Stamou et al. [5] cited the eighth observation in which a successful heart transplant was performed on a patient with isolated LVNC. An 18-year-old male patient, who had not previously been ill, was admitted at the Inova Fairfax Hospital for examination due to weakness, cough, and abdominal pain. Chest roentgenogram revealed cardiomegaly and pulmonary venous congestion. An echocardiogram demonstrated dilated cardiomyopathy with an ejection fraction less than 10%, mild mitral regurgitation and moderate tricuspid insufficiency, despite intravenous dobutamine treatment. The patient's condition progressively deteriorated. Repeat echocardiogram, unlike the first, revealed excessive trabeculations with deep recesses in the apex and in the middle third of the LV. For more than five months, the patient remained in the hospital on continuous intravenous inotropic support, and then he successfully underwent a heart transplant. The explanted heart weighed 426 g. Pathoanatomical examination confirmed isolated LVNC diagnosis. After heart transplantation, the patient was completely rehabilitated; he studied and worked for the next 2.5 years after surgery.

The ninth observation of heart transplantation in a non-compact myocardium was presented by J. Bordes et al. [16]. In this observation, noncompaction of the ventricular myocardium was associated with bicuspid aortic valve in a 42-year-old man who suffered from refractory acute heart failure and was successfully treated by heart transplantation. Prior to heart transplantation, transesophageal echocardiography revealed bicuspid aortic valve insufficiency, left ventricular dilatation with 40% ejection fraction. A surgical valve repair was proposed though the patient refused surgery. After 3 months, the patient was admitted to hospital with congestive heart failure in the intensive care unit. On admission (on dobutamine 20 mg/kg per minute), the heart rate was 89 beats per minute and blood pressure was 80/50 mmHg. Echocardiography showed severely depressed left ventricular systolic function with 25% ejection fraction. The LV was dilated with severe functional mitral regurgitation. Aortic regurgitation was measured minimal. Repeat echocardiography visualized multiple ventricular trabeculations in the LV, predominant to apical and mid-inferior areas. The maximal end systolic ratio of noncompacted to compacted layers was greater than 2. The RV appeared to be more heavily trabeculated than normal. Final clinical diagnosis: biventricular noncompaction. Subsequently, the patient underwent a heart transplantation three days after admission. Postoperative extracorporeal circulatory support was necessary for 24 hours. The patient was extubated on postoperative day 6. He was discharged from the intensive care unit on day 17 of post-transplantation and his condition remained satisfactory for the subsequent 2 months [16].

A 22-year-old Hispanic male presented with a two-month history of chest discomfort. Transthoracic echocardiogram revealed prominent trabeculae and spongiform appearance of the LV with 15–20% ejection-fraction, as well as similar changes in the right ventricular myocardium. Magnetic resonance imaging confirmed the echocardiogram data: excessive trabeculation of the left-ventricular apex and mid-ventricular segments, as well as at the apical and lateral wall of the RV. Cardiac catheterization showed an intact cardiac vessel system. The patient was placed on the heart transplant waitlist [17]. So, the young patient required a heart transplant due to severe heart failure resulting from biventricular noncompaction.

In some LVNC patients, a prolonged asymptomatic course is observed, while in others, progression of left ventricular systolic dysfunction is noted, resulting in death of patients if they have not undergone heart transplantation [4, 18]. In refractory heart failure developing in 4–12% of patients with noncompaction cardiomyo-

pathy, emergency heart transplantation is required [4]. However, this operation is largely limited due to shortage of donor organs [19].

MATERIALS AND METHODS

The case histories of three patients who presented with isolated LVNC were analyzed and the morphology of their ventricles that were removed during transplantation was studied in detail. Macroscopic and histological examination of the left ventricular anterior and lower walls, as well as the right ventricular free wall and interventricular septum were carried out.

After fixation with neutral formalin and dehydration, the material was poured into paraffin. Histological sections were stained with hematoxylin and eosin, and Masson's trichrome. PAS reaction was carried out and enclosed in Canadian balsam. Histological preparations were examined using a light microscope.

OWN CASES

Case No. 1

18-year-old female patient A.T.A. was admitted at Shumakov National Medical Research Center of Transplantology and Artificial Organs on November 10, 2009 presenting with shortness of breath with little physical activity and fatigue. ***Admission diagnosis:*** *Isolated LVNC syndrome, NYHA class 3, group 2 pulmonary hypertension, paroxysmal atrial fibrillation, paroxysmal supraventricular and ventricular tachycardia, incomplete left bundle branch block, UNOS status 1B.*

Anamnesis morbi. *She has been sick since the age of five. Until the age of 18, she was on the record of the Institute of Pediatrics, the USSR Academy of Medical Sciences. Appearance of paroxysmal supraventricular tachycardia has been noted since 1998. In 2007, there was an episode of acute left ventricular failure, arrested by diuretics and hormone therapy. In 2008, the patient was diagnosed with dilated cardiomyopathy, and in August 2009 she was diagnosed with isolated LVNC syndrome, NYHA class 3, and severe pulmonary hypertension. Therapy: dilantrend, prestarium, veroshpiron, furasemide, rhythmorm.*

Examination upon admission: *The patient's condition at admission was stable, moderately severe. Height 166 cm, body weight 43 kg. Pale skin. No peripheral edema. Lip cyanosis, acrocyanosis. No shortness of breath at rest. Vesicular breathing over the lungs, no wheezing. The heart borders are extended to the left by 1.5 cm from the mid-clavicular line. Arrhythmic heart sounds, systolic murmur at the apex and at the fifth point, extrasystole, heart rate of 90 beats per minute, and 85/50 mmHg blood pressure. Soft, painless abdomen. Liver and spleen are not enlarged. No focal neurological symptoms.*

Echocardiogram: left ventricular end-diastolic diameter (LVEDD) – 6.2 cm, left ventricular end systolic volume (LVESV) – 196, EF – 39–40%, pulmonary artery pressure (PAP) – 60 mmHg. Spherical heart with diffuse hypokinesis. A picture of left ventricular noncompaction with visualization of a narrow strip of up to 3 mm of compact myocardium.

Assessment of central hemodynamics: with no nitric oxide – heart rate – 91 bpm, blood pressure – 96/75/69 mmHg, pulmonary capillary wedge pressure (PCWP) – 33 mmHg, PAP – 72/53/38 mmHg, total pulmonary resistance (TPR) – 6.45 Wood units; use of functional samples of inhaled nitric oxide, heart rate – 96 bpm, blood pressure – 91/73/67 mmHg, PCWP – 18 mmHg, PAP – 36/28/20 mm Hg, TPR – 3.33 Wood units, which proved that pulmonary hypertension is reversible and that there are no contraindications for heart transplantation. Orthotopic heart transplantation was performed on November 26, 2009. Induction immunosuppression – Simulect 20 mg. The postoperative period was uneventful.

Pathoanatomical examination of native heart. Macroscopic examination results: *Heart ventricles are spherical in shape, isometric, excised along the atrioventricular groove, weighing 195 g, measuring 11.5 × 8 × 3.5 cm. Coronary arteries are wide, without stenosis. Fibrous ring of the mitral valve is 12 cm in perimeter. The left ventricular free wall is 0.8 cm thick, of which the compact layer is 2–3 mm thick. Severe left ventricular subendocardial fibrosis. Incised LV myocardium is brown, flabby, chord germination into the myocardium of the anterior left ventricular wall and part of the interventricular septum. The fibrous ring of the tricuspid valve is 13 cm in perimeter. Right ventricular free wall is 0.3 cm thick. Leaflets of the tricuspid valve are whitish in color.* ***Microscopic examination:*** *In the anterior left ventricular wall, in a thinned compact layer, severe diffuse albuminous degeneration and focal hydropic degeneration of cardiomyocytes. Individual cells are necrotic. In PAS reaction, uneven staining of the cytoplasm of cardiomyocytes (Fig. 1). Large thin-walled blood vessels are found in the anterior wall, and especially in the lower wall in the compact layer (Fig. 2). In the myocardial spongy layer, severe trabeculae endocardial sclerosis (Fig. 3). In the interventricular septum, excessive formation of fatty tissue and nerve trunks with perineural proliferation of connective tissue was noted (Fig. 4).* ***Pathoanatomical diagnosis:*** *Isolated left ventricular noncompaction with secondary LV dilation.*

Thus, heart failure was caused by isolated left ventricular noncompaction with secondary dilated left ventricle. Orthotopic heart transplantation was successfully performed on November 26, 2009. The patient was discharged from the clinic on December 29, 2009 in a satisfactory condition.

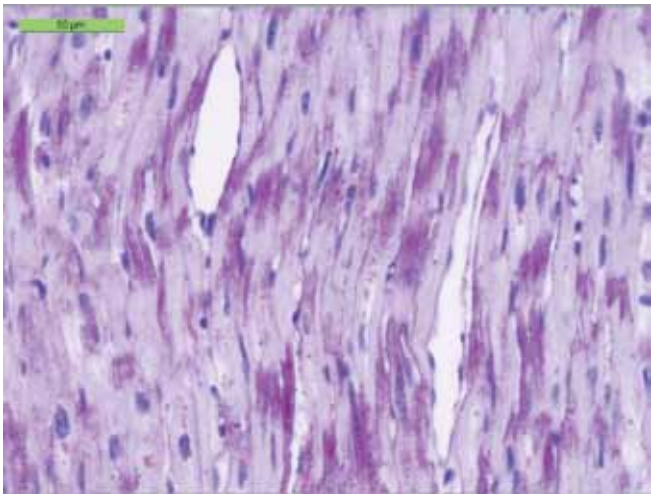

Fig. 1. Non-uniform PAS-positive colouring of the cytoplasm of the cardiac myocytes in a anterior wall of the left ventricle. PAS stain. ×400

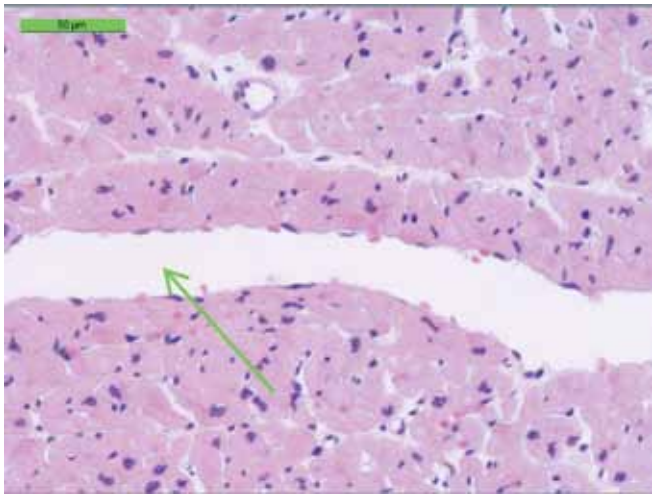

Fig. 2. Large thin-walled blood vessel (green arrow) in a compact layer of the inferior wall of the left ventricle. H & E stain. ×400

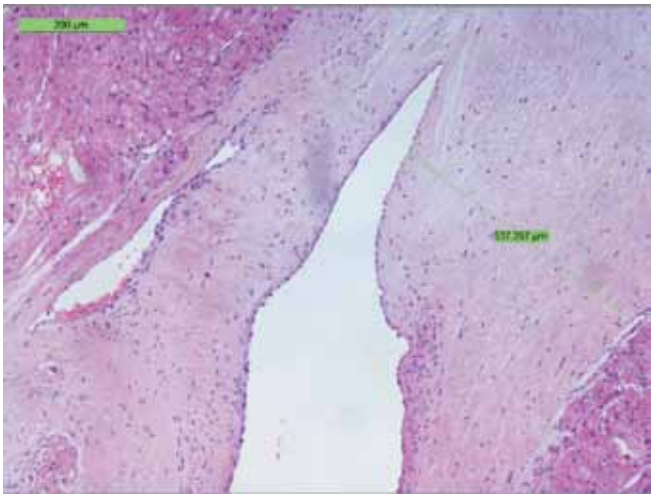

Fig. 3. The expressed sclerosis of the endocardium of the trabeculae in the field of a anterior wall of the left ventricle. H & E stain. ×100

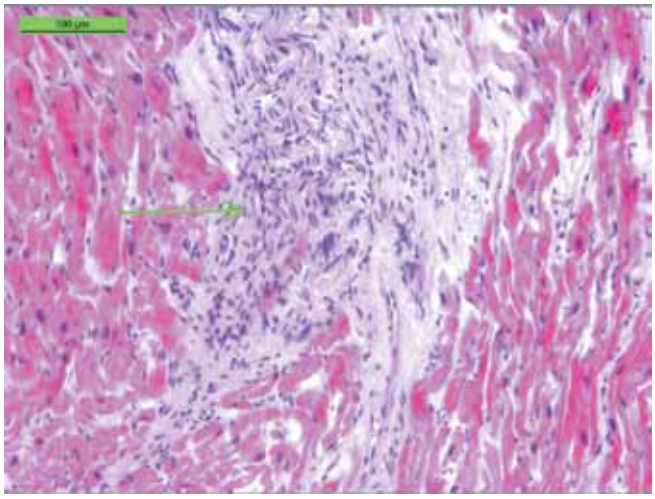

Fig. 4. Nervous fulcrum of the vegetative system in the interventricular septum. H & E stain. ×200

Case No. 2

49-year-old female patient I.E.V. was admitted at Shumakov National Medical Research Center of Transplantology and Artificial Organs, presenting with complaints of weakness, shortness of breath at rest and on minimum exertion, enlarged abdomen, leg swelling. ***Admission diagnosis. Underlying condition:*** *Restrictive cardiomyopathy.* ***Complications of underlying condition:*** *Low left ventricular ejection fraction. Circulatory failure (third degree), NYHA class 3–4.*

Anamnesis morbi. *For a long time, various abnormal heart rhythm, mainly atrial, were observed. Radiofrequency ablation (RFA) – no effect. In 2011, Cox Maze procedure, right atrial isolation, and implantation of epicardial pacemaker were performed. Ascites and edema appeared from September 2012. Shortness of breath for the last three months. Against the background of diuretic therapy, the rate of diuresis decreased, azotemia progressed. Echocardiogram in May 2013 revealed small left ventricular volumes, enlargement of the right parts without signs of pulmonary hypertension. Due to hyperkalemia and azotemia, hemodialysis and ultrafiltration were performed in May-June 2013. The patient was admitted for examination according to heart transplantation program. Oligoanuria persisted, daily hemodiafiltration (HDF) sessions were held.*

Examination upon admission: *Severe condition. Fully conscious. Moves on a chair. Walks with difficulty. Skin with bronze tint, dry, decrease in skin turgor. Swelling of leg, feet and hips. Ascites. Auscultatory vesicular breathing, weakened in the lower parts of the lungs. No wheezing. Respiratory rate = 18 per minute. Rhythmic pulse of reduced filling and tension, without deficiency, heart rate = 90 per minute. Heart sounds are muffled.*

Blood pressure 100/70 mmHg. Abdominal swelling due to unstressed ascites. Liver and kidneys are not palpable. Negative pasternatsky symptom. No dysuric symptoms.

Echocardiogram: LVEDD – 5.4 cm, LVESV – 176, EF – 20%, PAP – 30 mmHg.

Assessment of central hemodynamics: with no nitric oxide – heart rate – 91 bpm, blood pressure – 96/75/69 mmHg, PCWP – 33 mmHg, PAP – 31/22/18 mmHg, TPR – 3.1 Wood units.

The patient was prepared for heart transplant surgery. ***Pre-transplant diagnosis. Underlying condition:*** *Restrictive cardiomyopathy.* ***Complications of underlying condition:*** *Low left ventricular ejection fraction. Circulatory failure (third degree), NYHA class 3–4. UNOS status 1B.* ***Coexisting conditions:*** *Right-sided nephroptosis. Right kidney cyst. Nephropathy. Condition after HDF sessions.*

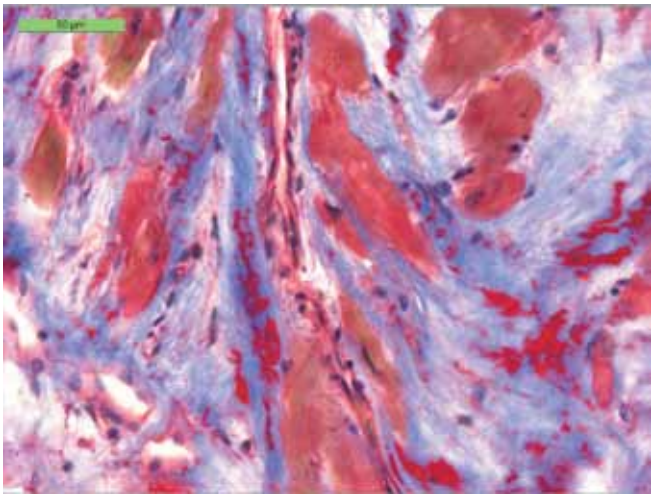

Fig. 5. Dystrophy of the cardiac myocytes and sclerosis of the interstitium with degeneration of the collagen in the inferior wall of the left ventricle. Masson trichrome stain. ×400

Orthotopic heart transplantation with reimplantation of epicardial pacemaker was performed on September 17, 2013.

Pathoanatomical examination of native heart. Macroscopic examination results: *Heart ventricles are excised along the atrioventricular groove, weighing 285 g, measuring 12 × 10.5 × 4 cm. Coronary arteries are wide. Intima of the coronary arteries and their branches are smooth, clean, the arteries are not narrowed throughout. Fibrous ring of the mitral valve is 12 cm in perimeter. Left ventricular wall in the middle sections is 1.2 cm thick. Incised myocardium is brown, in the middle sections of the anterior left ventricular wall with a partial transition to the interventricular septum and a 3 mm thick compact layer in the apex area. The spongy layer reaches 8–10 mm in thickness, deep and wide slits between the trabeculae. The fibrous ring of the tricuspid valve is 10.5 cm in perimeter. The right ventricular wall is 0.4 cm thick. The interventricular septum is 1 cm thick.*

Microscopic examination: *In the anterior and lower left ventricular wall, there is severe degeneration, chaotic and disorderly arrangement of cardiomyocytes in the compact myocardial layer; diffuse focal interstitium sclerosis with collagen degeneration (Fig. 5). The spongy layer also consists of multiple trabeculae with wide intertrabecular spaces (Fig. 6), mild endocardial sclerosis and focal subendocardial fibrosis (Fig. 7). Collagen degeneration was noted in some trabeculae of the anterior left ventricular wall, as in the compact layer (Fig. 8). In the interventricular septum – cardiomyocyte degeneration, diffuse interstitial sclerosis and proliferation of connective tissue around the vessels. In the right ventricular wall, there is degeneration of cardiomyocytes, many hypertrophied cells, as well as a chaotic and disorderly arrangement of cardiomyocytes. Intramyocardial vessels and epicardial vessels – with no*

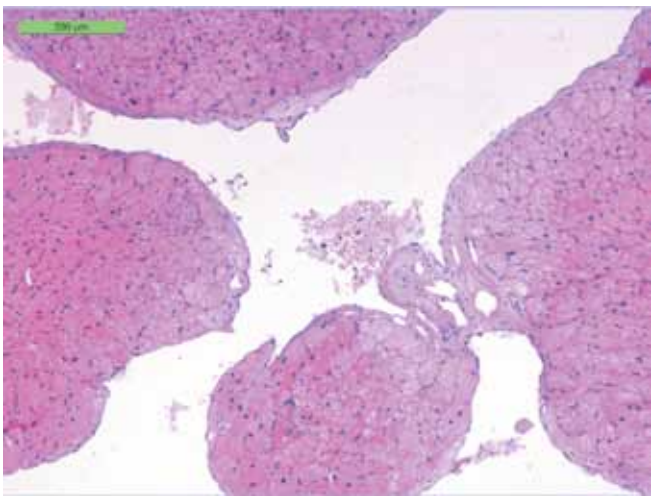

Fig. 6. Trabeculas with wide intertrabeculas spaces in a anterior wall of the left ventricle. H & E stain. ×100

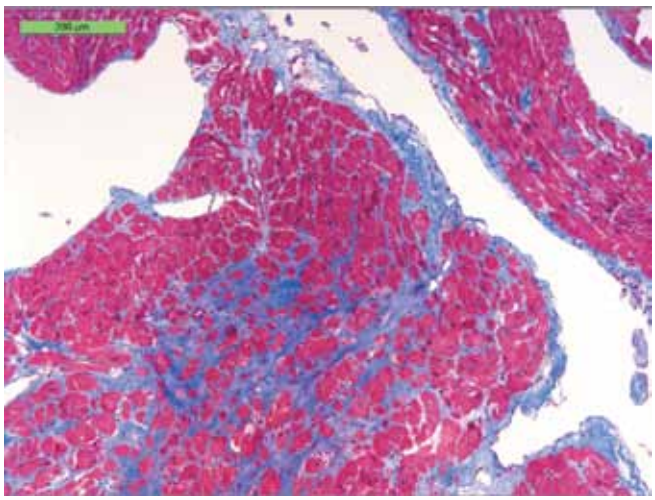

Fig. 7. Diffuse sclerosis of the trabeculas in a anterior wall of the left ventricle. Masson trichrome stain. ×100

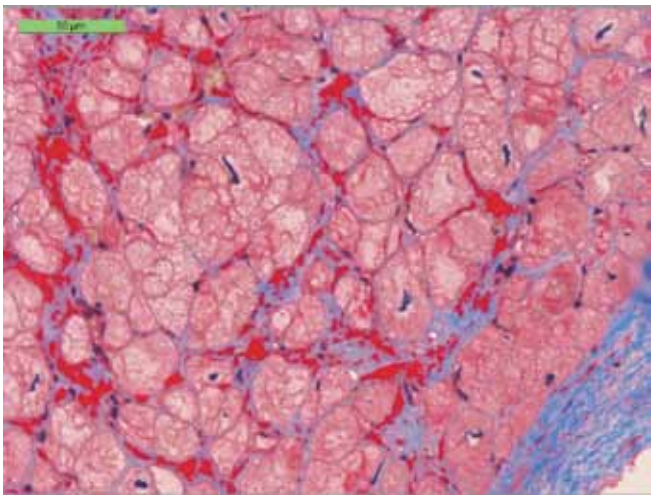

Fig. 8. Dystrophy of the cardiac myocytes and a degeneration of collagen in interstitium of the trabeculas in the free wall of the right ventricle. Masson trichrome stain. ×400

abnormalities. ***Pathoanatomical diagnosis****: Isolated left ventricular noncompaction.*

The patient was transferred from intensive care unit to the ward on day 14 of the postoperative period. ***Definitive clinical diagnosis. Underlying condition:*** *Isolated left ventricular noncompaction.* ***Surgery****. Orthotopic heart transplantation performed on September 17, 2013.* ***Complications of underlying condition:*** *Circulatory failure (first degree), NYHA class 2. Mild iron-deficiency anemia. Condition after HDF sessions.* ***Coexisting conditions:*** *Right-sided nephroptosis. Right kidney cyst. The patient underwent standard therapy.*

Upon discharge on October 30, 2013 and to the present time, the patient's condition has been stable. No complaints.

Case No. 3

31-year-old male patient H.G.M. was admitted at Shumakov National Medical Research Center of Transplantology and Artificial Organs on November 11, 2015 presenting with shortness of breath with minimal physical activity, nocturnal choking episodes, heart failure, weakness.

Anamnesis morbi. *In 2007, when examining the patient for syncope attacks, dilated cardiomyopathy was diagnosed, abnormal heart rhythm was detected as paroxysmal supraventricular tachycardia and paroxysmal unstable ventricular tachycardia. Treatment was performed with beta-blockers, aldosterone antagonists, amiodarone 200 mg per day, disaggregants with no pronounced clinical effect. Deterioration within 2 months, when the signs of heart failure began to progress against the background of drug therapy.*

Admission diagnosis. Underlying condition: *Noncompaction cardiomyopathy.* ***Complications of underlying condition:*** *Right-sided hydrothorax. Chronic heart failure (diastolic). NYHA class III. Abnormal heart rhythm: paroxysmal unstable ventricular tachycardia and paroxysmal supraventricular tachycardia.*

Examination upon admission: *Severe condition due to heart failure. Fully conscious. Can talk. A clear skin, acrocyanosis. Swollen leg and feet. Peripheral lymph nodes are not enlarged. Borders of the heart are expanded to the left by percussion. Heart sounds are muffled, normal rhythm, heart rate is 80 beats per minute. Blood pressure 100/70 mmHg. Pulsation in the arteries of both feet are preserved. Normal chest shape. Borders of the lungs are unchanged. Breathing is free, stiff, occurring in all lobes, weakened in the lower lateral lobes, more on the right, no wheezing. Respiratory rate 24 per min. Tongue is wet, covered with white coating. Abdomen on palpation is soft, painless. Liver +4 cm outward from the edge of the costal arch. No dysuric disorders.*

Echocardiogram: LVEDD – 5.8 cm, LVESV – 179, EF – 25%, PAP – 35 mmHg. Pronounced trabeculation of the cavity and blood clot in the left ventricular apex region, pronounced diffuse hypokinesis.

Spiral CT scan revealed expansion of the heart chambers, blood clots in the projection of the apices of the left and right ventricles, right-sided hydrothorax with compression of the right lung, and also signs of congested liver.

Assessment of central hemodynamics: with no nitric oxide – heart rate – 98 bpm, blood pressure – 99/73/65 mmHg, PCWP – 8 mmHg, PAP – 20/10/15 mmHg, TPR – 1.3 Wood units.

Orthotopic cardiac allotransplantation was performed a week after admission – November 10, 2015.

Pathoanatomical examination of native heart. Macroscopic examination results: *Heart ventricles are excised along the atrioventricular groove, weighing 320 g, measuring 15 × 15 × 4 cm. Intima of the trunk and branches of the left and right coronary arteries are smooth and shiny.*

Fibrous ring of the mitral valve is 13 cm in perimeter. Left ventricular cavity is dilated. Its wall thickness varies from 0.9 to 1.3 cm at various levels. With the exception of the basal sections, there is almost no compact layer, consisting of a trabecular spongy layer. In the intertrabecular spaces of the lower and middle thirds, especially in the apex region, multiple clots of varying degrees of formation are visible, mottled from red to gray (Fig. 9). The right ventricular cavity is also dilated. The fibrous ring of the tricuspid valve is 15.5 cm in perimeter, and the myocardium is 0.1 to 0.2 cm thick. The interventricular septum is 1.1 cm thick, adipose tissue layers are visible from the cut section. ***Microscopic examination:*** *In the anterior and lower left ventricular wall, the myocardium compact layer is less than 1 mm (Fig. 10). The spongy layer includes multiple trabeculae with wide intertrabecular spaces. Trabeculae with severe sclerosis (Fig. 11), degeneration and deterioration of*

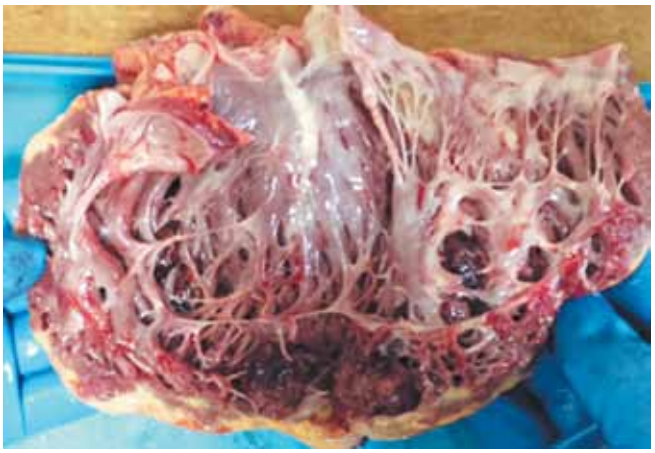

Fig. 9. Removed ventricels of the native heart of the recipient. In figure – an opened cavity of the left ventricle of the heart. Dilated his cavities. In a free wall of the ventricle almost completely there is no compact layer of a myocardium

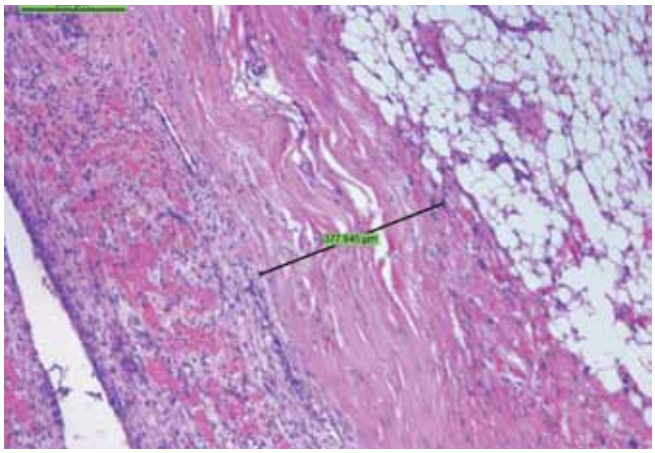

Fig. 10. Extremely thin a compact layer of the inferior wall of the left ventricle. H & E stain. ×100

cardiomyocytes (Fig. 12). In the right ventricular free wall, there is also interstitial sclerosis with collagen and cardiomyocyte degeneration. In the interventricular septum, myocardial sclerosis is less pronounced. At the same time, sympathetic trunks of the autonomic nervous system appear to be enclosed in the connective tissue layers. Along with connective tissue proliferation in the interventricular septum, a significant content of fatty tissue was noted (Fig. 13). ***Pathoanatomical diagnosis:*** *Isolated left ventricular noncompaction.*

Since the patient was discharge on December 15, 2015, and to the present time, his general condition and graft function have remained satisfactory. No complaints.

DISCUSSION

At our research center, patients who have congestive heart failure caused by dilated or ischemic cardiomyo-

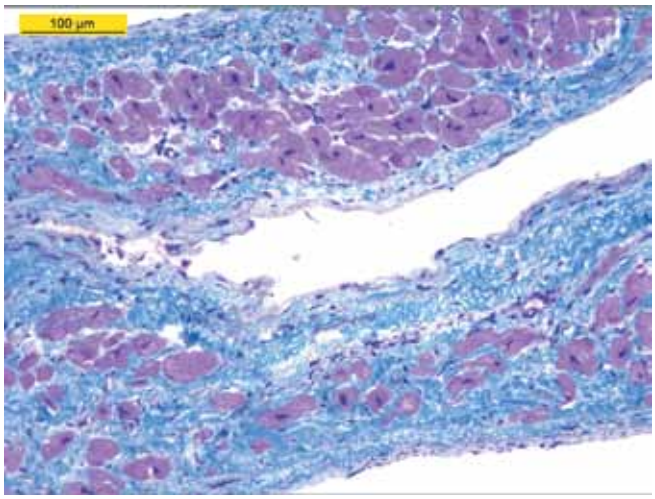

Fig. 11. The expressed sclerosis of the endocardium and of the interstitium, and also an atrophy of the cardiac myocytes of the trabeculas in the anterior wall of the left ventricle. Masson trichrome stain. ×200

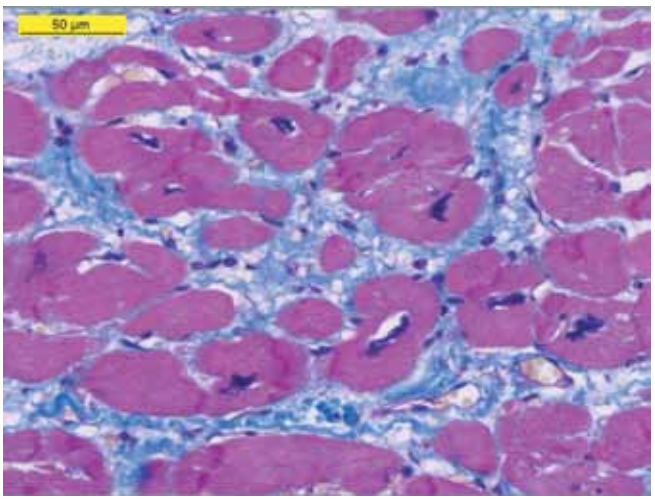

Fig. 12. Ttrabecula in a anterior wall of the left ventricle. The expressed dystrophy of the cardiac myocytes with perinuclear edema and hyperchromatosis of the misshapen nucleus. Masson trichrome stain. ×400

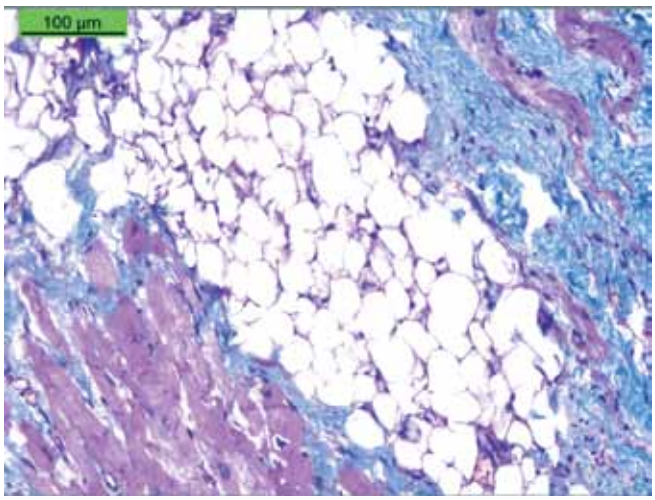

Fig. 13. Fatty tissue and sclerosis of the interstitium in the interventricular septum. Masson trichrome stain. ×200

pathy are the main group in the heart transplant waiting list. However, over the past decade, heart transplant has been performed for patients suffering from other heart diseases, particularly, such a rare condition as isolated LVNC. From 1986 to December 25, 2019, about 1,413 heart transplants were performed at our research center. Isolated non-compaction of the left ventricular myocardium was diagnosed via echocardiogram and spiral CT scan and morphologically confirmed in only three patients, which is only 0.2%. Based on available reports [4, 5, 16, 17], only 10 cases of heart transplant in adults suffering from isolated LVNC have been published to date.

The difficulty in diagnosing isolated LVNC, which many authors discuss [6–9], can be noted in the cases we have cited. Two patients (cases 1 and 3) were for a long time diagnosed with dilated cardiomyopathy. It was only at the stage of development of end-stage heart failure in one patient (case 1) that the right clinical diagnosis was made before heart transplantation. In the other patient (case 3), right clinical diagnosis was made only during hospitalization at our research center. One of the patients (case 2), right up to heart transplantation, was diagnosed with restrictive cardiomyopathy. The correct diagnosis, isolated LVNC, was made only after morphological examination.

According to some reports [6–8], it is necessary to conduct a particularly thorough differential diagnosis of isolated LVNC with dilated cardiomyopathy, in which dilatation of the left ventricular cavity is accompanied by a thinning of the myocardium compact layer, which gives the impression of hypertrabeculation. On the contrary, with isolated LVNC, left ventricular cavity dilatation can lead to erroneous diagnosis of dilated cardiomyopathy [6]. W.C. Roberts et al. [7], reporting about cardiac transplantation done in 3 patients (2 women), aged 36, 45, and 49 years for what was diagnosed clinically as nonischemic dilated cardiomyopathy, noted hypertrabeculation involving the free wall of the dilated left ventricle. In their own observations, the authors talk about isolated LVNC. However, they believe that analysis of gross photographs of the heart from previous publications by other authors suggests that isolated LVNC is overdiagnosed at least morphologically [7]. This thesis is confirmed by M. Zhang et al. [8], who examined 64 native heart transplant patients with a clinical diagnosis of dilated cardiomyopathy; five of these patients had a mildly noncompacted left ventricle with hypertrabeculation.

Patients with isolated LVNC rarely need a heart transplant surgery. This is determined by the severity of refractory congestive heart failure. It can occur in infants and may be absent even in adults. Moreover, many patients with isolated LVNC are asymptomatic [4, 18]. In all the three heart transplant cases at our research center, the patients suffered from congestive heart failure associated with isolated LVNC.

Preparation for surgery, heart transplant technique and post-transplantation management of patients suffering from isolated LVNC do not differ from the general cohort of transplant patients, but nevertheless require a personalized approach.

CONCLUSION

Isolated left ventricular noncompaction is a rare condition that has not been well publicized in scientific literature. It can occur without symptoms and cause severe refractory congestive heart failure that necessitates a heart transplant surgery.

The authors declare no conflict of interest.

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MICRORNA EXPRESSION LEVELS IN EARLY AND LONG-TERM PERIOD FOLLOWING HEART TRANSPLANTATION

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Objective: to conduct comparative analysis of the expression levels of microRNA-101, microRNA-142, microRNA-27, microRNA-339 and microRNA-424 in patients with severe chronic heart failure and in heart recipients in the early and long-term period following heart transplantation and to determine the association with acute transplant rejection. **Materials and methods.** The study included 46 heart recipients, among whom were 36 men (78.3%); the average age of the recipients was 47.7 ± 10.8 (16 to 67) years, and 12 patients with end-stage chronic heart failure, among whom were 8 men (66.7%); the average age of the patients was 46.1 ± 6.4 (37 to 64) years. The control group consisted of 12 healthy individuals, not significantly different by gender and age. microRNA expression levels in blood plasma were determined through quantitative polymerase chain reaction (Q-PCR). Transplant rejection was verified via morphological analysis of endomyocardial biopsy specimens. **Results.** Blood plasma of patients with end-stage chronic heart failure had significantly higher expression rates of microRNA-101, microRNA-27, microRNA-339 and microRNA-424 than in healthy individuals ($p < 0.02$). In the early stages following transplantation, the expression levels of microRNA-101 and microRNA-27 in heart recipients were significantly lower than in patients with severe chronic heart failure ($p < 0.003$). A year or more after transplantation, there were no significant differences in the expression levels of microRNA-101, microRNA-142, and microRNA-339 in heart recipients and in healthy individuals. In recipients with acute rejection, the expression levels of microRNA-101 and microRNA-27 significantly differed from that of recipients without signs of rejection ($p = 0.04$ and $p = 0.03$, respectively). **Conclusion.** The obtained data on changes in the expression levels of microRNA-101 and microRNA-27 in heart recipients with acute transplant rejection suggests possible diagnostic value of these biomarkers in determining the risk of rejection.

Keywords: heart transplantation, microRNA, chronic heart failure, biomarkers, rejection.

Significant advances in surgical techniques and improved immunosuppressive therapy have increased survival and improved quality of life for heart recipients. The most important task in managing patients after transplantation is to prevent graft rejection along with minimizing the dose of immunosuppressive drugs. Development of non-invasive methods for detecting transplant rejection will improve early diagnosis and increase life expectancy by reducing the number of late post-transplant complications [1–3].

In recent years, a number of biomarkers have been shown to be involved in cardiovascular complications in patients with heart failure and in heart transplant recipients. It has been demonstrated that an estimate of concentration of these biomarkers can be used to predict and diagnose heart transplant rejection [4–6]. A separate group consists of microRNAs – small, non-coding RNAs that regulate gene expression. Ability to accurately and quickly determine microRNA content in biological fluids in combination with their tissue and nosological specificity makes these small signaling molecules promising candidates for the role of rejection biomarkers in heart transplant recipients [7–9].

In the present work, we performed a comparative analysis of the expression levels of miRNA-27, miRNA-101, miRNA-142, miRNA-339 and miRNA-424 in heart recipients in early and long-term follow-up after transplantation and determined the relationship with acute graft rejection.

MATERIALS AND METHODS

The study included 46 patients who, in the period from 2013 to 2016, at Shumakov National Medical Research Center of Transplantology and Artificial Organs,

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underwent a heart transplant (HT) surgery. Among them were 36 men (78.3%); average age of the recipients was 47.7 ± 10.8 (16 to 67) years. In the study were also 12 patients with severe chronic heart failure (NYHA class III and IV), 8 of them were men (66.7%); average age of the patients was 46.1 ± 6.4 (37 to 64) years. Dilated cardiomyopathy (DCM) was diagnosed in 29 (63%) recipients before HT. Coronary heart disease (CHD) was diagnosed in 12 (26%) recipients before HT, while other complications were found in 5 (11%) recipients. The recipients were observed for a maximum of 2215 days (median 264.5 [32; 785.3]) after HT. The control group consisted of 12 healthy persons, who did not significantly differ by sex and age.

All patients who had indications for HT were routinely examined in accordance with the national clinical recommendations "Heart Transplantation and Mechanical Circulatory Support" and the patient management protocol at Shumakov National Medical Research Center of Transplantology and Artificial Organs. After transplantation, scheduled recipient examinations included: clinical evaluation of the condition, general and biochemical blood tests (including measurement of tacrolimus blood concentrations), 24-hour blood pressure monitoring (to correct antihypertensive therapy), echocardiography, repeated myocardial biopsies, and annual coronary angiography.

All recipients received a three-component immunosuppressive therapy, including a combination of calcineurin inhibitors (tacrolimus) and cytostatic inhibitors (mycophenolate mofetil or mycophenolic acid), as well as varying doses of prednisolone orally (depending on the time after surgery and the frequency of episodes of graft rejection) and adjuvant therapy, if indicated [10].

Acute heart transplant rejection was diagnosed based on histological examinations and humoral and immunohistochemical tests of endomyocardial biopsy specimens.

Venous blood plasma served as the material for studying miRNA expression; in heart recipients, 56 samples were studied at various times after transplantation (1 to 3 samples from each patient, average of 1.22).

Total RNA isolation from peripheral blood plasma

Peripheral blood samples of patients were collected in disposable tubes with an EDTA anticoagulant, centrifuged for 10 minutes at 3000 rpm. Blood plasma was separated from the cell pellet and immediately frozen at –200 °C. RNA was isolated from 100μL blood plasma using Serum Plasma kits (Qiagen, USA) with preliminary addition of 1.6 x10^8 copies of synthetic miRNA cel-miR-39 (Qiagen) after plasma incubation with Qiazol phenolic mixture. Cel-miR-39 was used as an internal control to check RNA isolation efficiency, complementary DNA (cDNA) synthesis and real-time polymerase chain reaction (PCR).

Real-Time quantitative reverse transcription PCR

Total RNA from each sample was converted to cDNA in a reaction mixture (20μL) containing 1xmi Script HiSpec Buffer, 1xmi Script Nucleics Mix, at T = 37 °C for 60 minutes, followed by incubation at 95 °C for 5 minutes, cooling on ice and bringing the sample volume to 200 μL with deionized water. Synthesized cDNA (2 μL) was the matrix in real-time PCR using primers specific for the studied miRNAs: miRNA-27, miRNA-101, miRNA-142, miRNA-339, miRNA-424, Ce_miR-39 (miScript Primer assay, Ce_miR-39_1, Qiagen), and the miScript SYBR Green PCR Kit (Qiagen). PCR reaction conditions: 15 minutes at T = 95 °C followed by 40 cycles of 15 seconds at T = 94 °C, 30 seconds at T = 50 °C and 30 seconds at T = 70 °C in a CFX 96 amplifier (Biorad). MicroRNA expression intensity was calculated using the $2^{-\Delta Ct}$ method [11] and was expressed in relative units equivalent to $\log_2 (2^{-\Delta Ct})$, where ΔCt are the working values of change in the product production cycle relative to the internal control of miRNA (Ce_miR-39) expression.

Statistical data processing

Statistical analysis of results was done using application software package IBM SPSS STATISTICS 20 (IBM SPSS Inc., USA). Statistical processing of obtained data was carried out by nonparametric methods: Wilcoxon signed-rank test was used to compare dependent samples, while the Mann-Whitney U test was used to compare independent variables. The critical significance level was taken to be 5%, i.e. the null hypothesis was rejected at $p < 0.05$.

RESULTS

In patients with end-stage chronic heart failure, as well as in heart transplant recipients, miRNA expression indicators varied over a wide range. They exhibited a nonparametric distribution. In this paper, results are represented by median and interquartile range, expressed in relative units.

Expression levels of miRNA-101, miRNA-142, miRNA-27, miRNA-339 and miRNA-424 in patients with severe chronic heart failure and heart transplant recipients did not significantly differ in men and women ($p = 0.29$, $p = 0.33$, $p = 0.25$, $p = 0.71$ and $p = 0.07$, respectively). MicroRNA expression indicators were not dependent on age.

A comparative analysis of miRNA expression revealed significantly higher expression levels of miRNA-101, miRNA-27, miRNA-339 and miRNA-424 in

the blood plasma of patients with end-stage chronic heart failure compared with the healthy persons (Fig. 1).

The established differences in the expression levels of miRNA-101, miRNA-27, miRNA-339 and miRNA-424 probably reflect the totality of pathological conditions in the myocardium of patients with severe chronic heart failure.

No significant differences in the expression levels of miRNA-101, miRNA-142, miRNA-27, miRNA-339 and miRNA-424 were found in heart transplant recipients depending on the initial diagnosis, serving as an indication for transplantation: DCM or CHD ($p = 0.89$, $p = 0.44$, $p = 0.87$, $p = 0.08$ and $p = 0.52$, respectively).

There was no reliable correlation between the expression level of miRNA-101 ($r = 0.001$, $p = 0.99$), miRNA-142 ($r = 0.004$, $p = 0.98$), miRNA-27 ($r = -0.06$, $p = 0.68$), miRNA-339 ($r = 0.06$, $p = 0.7$) and miRNA-424 ($r = 0.03$, $p = 0.84$) and tacrolimus blood concentrations in heart transplant recipients (Fig. 2).

The results of comparative analysis of miRNA expression indicators in patients with severe heart failure and in heart transplant recipients are presented in Table 1.

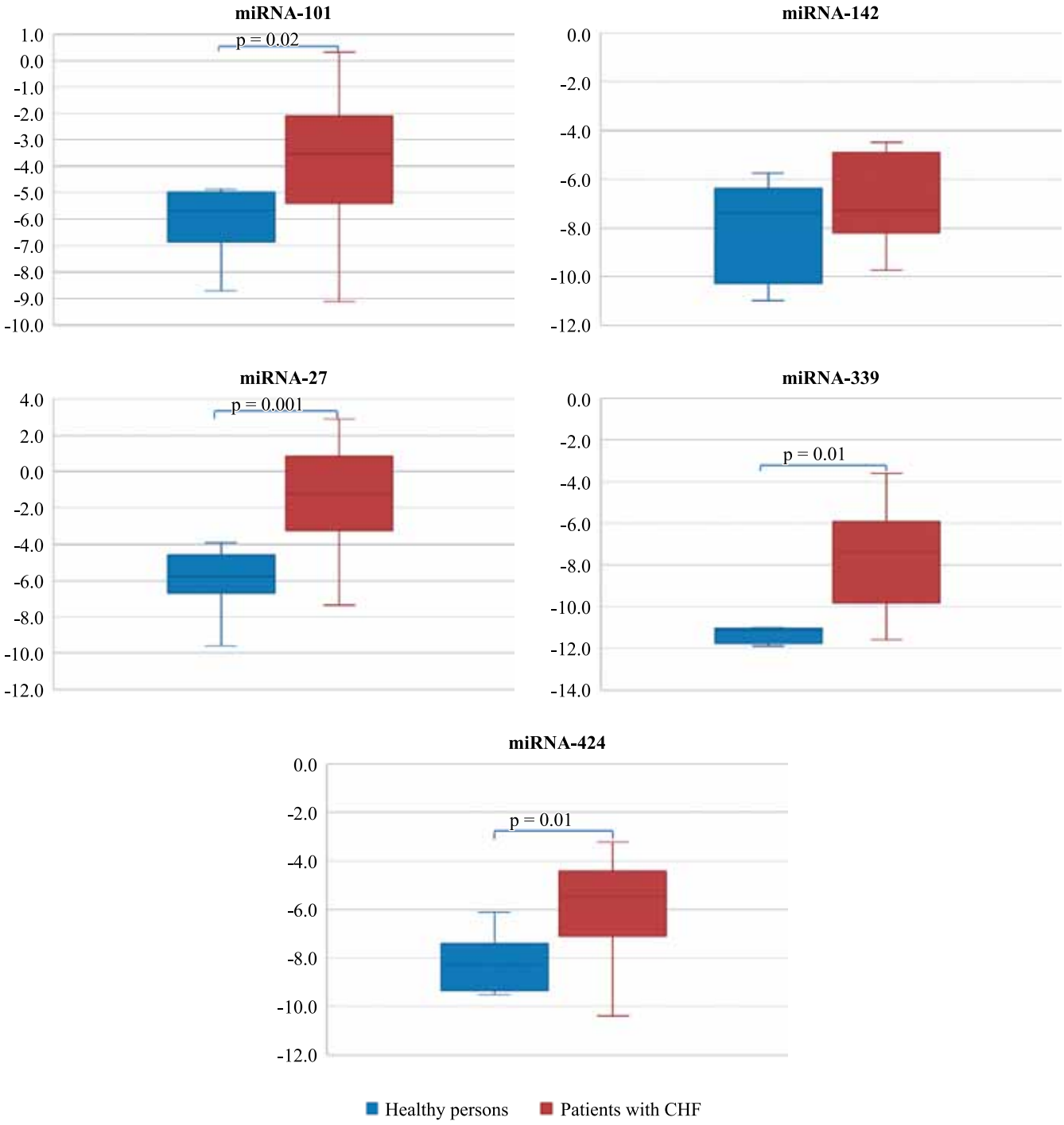

Fig. 1. miRNA expression levels in healthy persons and in patients with severe chronic heart failure, $\log_2(2^{-\Delta Ct})$

Table 1

Comparative analysis of miRNA expression in patients with chronic heart failure and in heart recipients

microRNA, $\log_2(2^{-\Delta Ct})$	CHF patients	Heart recipients	Significance, p
miRNA-101	–3.53 [–5.4; –2.09]	–7.21 [–8.98; –5.1]	**0.0002**
miRNA-142	–7.27 [–8.2; –4.9]	–6.67 [–8.19; –5.5]	0.67
miRNA-27	–1.23 [–3.26; 0.84]	–4.78 [–5.94; –2.88]	**0.01**
miRNA-339	–7.38 [–9.81; –5.9]	–10.13 [–11.59; –9.02]	**0.04**
miRNA-424	–5.46 [–7.11; –4.42]	–7.05 [–8.11; –5.81]	0.52

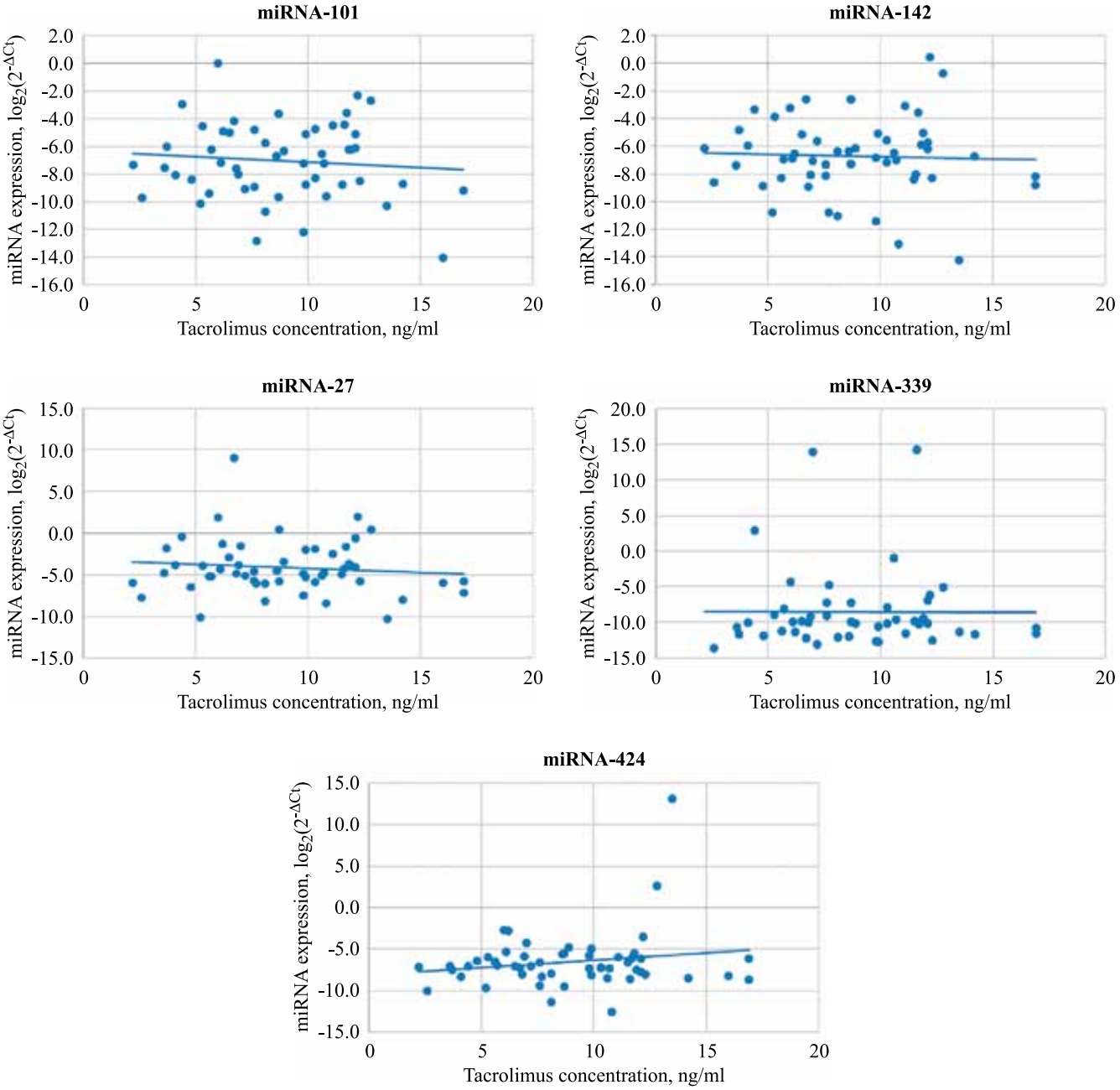

Fig. 2. Analysis of correlation between miRNA expression levels and tacrolimus concentration

Groups of patients with severe chronic heart failure and heart transplant recipients did not significantly differ by gender and age.

The differences in the expression levels of miRNA-101, miRNA-27, miRNA-339 in the groups of potential heart recipients and all patients included in the study after transplantation were significant (p = 0.0002, p = 0.01 and p = 0.04, respectively).

It was found that in the early post-transplant period (median 24 [10; 35] days), the expression levels of miRNA-142, miRNA-339 and miRNA-424 in heart recipients did not differ significantly from patients with severe chronic heart failure, although there was a tendency towards reduction in the levels. Differences in the expression levels of miRNA-101 and miRNA-27 in these groups were significant (p = 0.0001 and p = 0.003, respectively). Changes in the expression profile of miRNA-101 and miRNA-27 in the early post-transplant period may be due to the action of a complex of various factors associated with surgical intervention, including systemic inflammatory response, adaptation of the body to the transplanted organ, and immunosuppressive therapy. There is evidence that these signaling molecules are involved in regulation of myocardial fibrosis through interactions with transcription factor RUNX1 and transforming growth factor-beta receptor (TGFβR1) [12, 13].

Comparative analysis of miRNA expression in heart recipients in the early and long-term follow-up after transplantation is presented in table 2.

In heart recipients, expression levels of miRNA-142, miRNA-339 and miRNA-424 a year or more after transplantation did not significantly differ compared with recipients in the early stages after HT, but there was a tendency towards increase in the levels. Differences in the expression levels of miRNA-101 and miRNA-27 in these groups were significant (p = 0.008 and p = 0.04, respectively).

Fig. 3 shows miRNA expression indicators in heart transplant recipients in the early and long-term post-transplant periods and in healthy persons.

It was found that a year or more after transplantation, the expression levels of miRNA-101, miRNA-142 and miRNA-339 in heart recipients did not significantly differ from those of healthy persons. Differences in the expression levels of miRNA-27 and miRNA-424 in these groups were significant (p = 0.003 and p = 0.01, respectively).

Analysis of the effect of acute graft rejection on expression level of the studied miRNAs revealed the following. During the entire follow-up period after transplantation, signs of acute graft rejection were verified in 27 recipients through 31 endomyocardial biopsy samples. Among them were acute cellular rejection (R1G–R3G according to ISHLT-2004 grading system) was observed in 23 recipients in 24 samples, humoral rejection in 6 recipients in 6 samples and mixed rejection in one sample (Table 3).

Recipients with and without acute graft rejection did not significantly differ by age, gender, and pre-transplant diagnosis. Analysis of tacrolimus blood concentration in heart recipients revealed no significant differences in the group of patients with and without acute graft rejection. The concentration levels were 8.1 [6.7; 10.7] and 9.9 [6.1; 12] ng/ml, respectively (p = 0.75).

Table 4 presents a comparative analysis of miRNA expression in heart recipients with and without acute graft rejection.

Significant differences were found in the expression values of miRNA-101 and miRNA-27 in recipients with acute graft rejection compared with recipients without rejection (p = 0.04 and p = 0.03, respectively). The results confirm the available data on the possible diagnostic role of miRNAs, particularly miRNA-101, in acute heart transplant rejection [14].

Table 2

Comparative analysis of miRNA expression in heart transplant recipients in the early and long-term post-transplant periods

microRNA, $\log_2(2^{-\Delta Ct})$	Post-HT period		Significance, p
	1 month, n = 22	1 year or more, n = 34	
miRNA-101	–8.75 [–9.74; –6.76]	–6.22 [–7.59; –4.78]	**0.008**
miRNA-142	–7.03 [–8.35; –6.01]	–6.52 [–7.39; –5.09]	0.25
miRNA-27	–5.79 [–6.06; –4.61]	–4.08 [–5.07; –1.92]	**0.04**
miRNA-339	–10.61 [–11.73; –9.93]	–9.88 [–11.37; –8.15]	0.83
miRNA-424	–7.13 [–8.15; –6.13]	–6.99 [–8.01;–5.74]	0.45

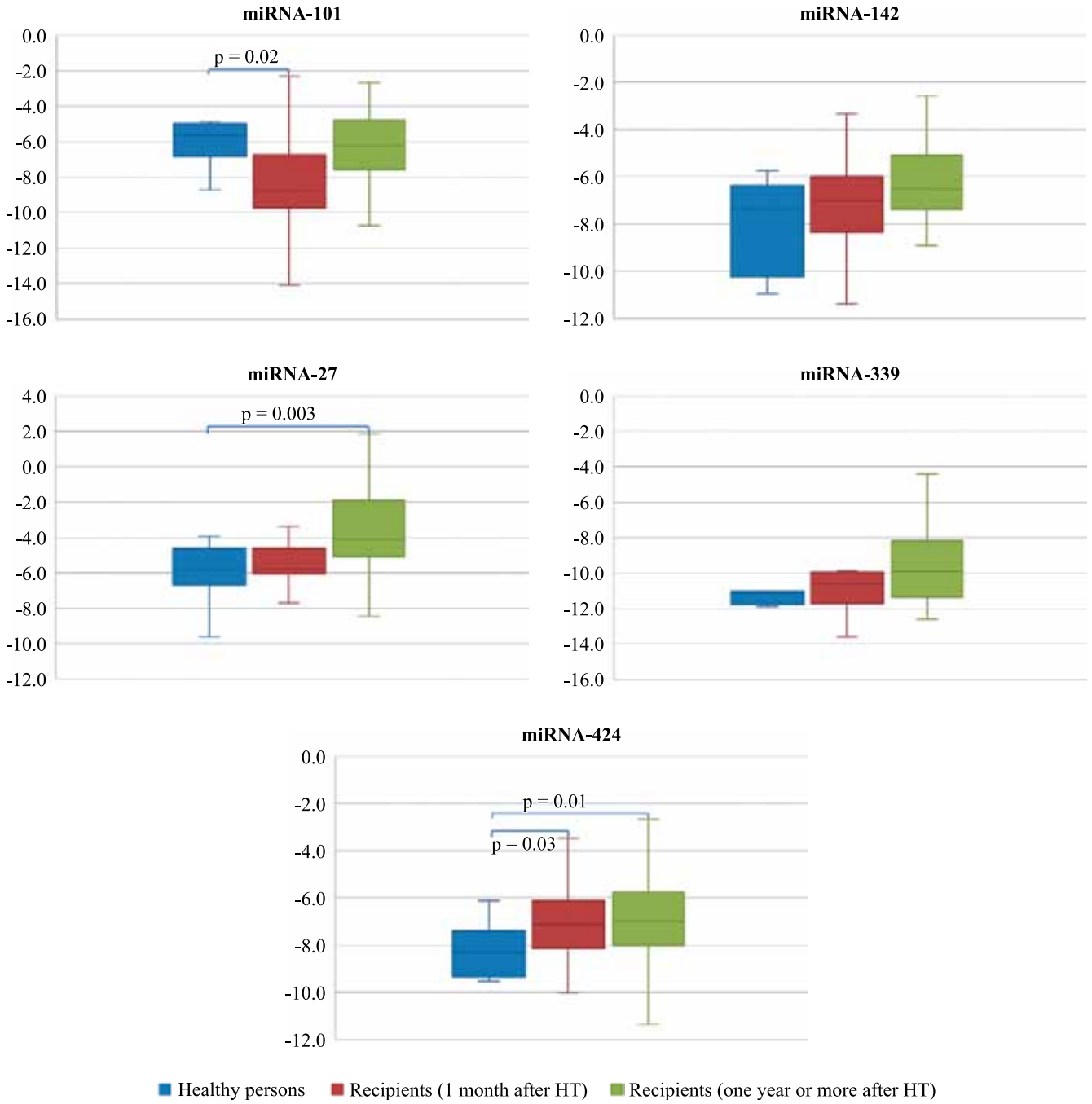

Fig. 3. miRNA expression levels in heart recipients in the early and long-term post-transplant periods, $\log_2(2^{-\Delta Ct})$

Table 3

Characteristics of heart transplant recipients with and without rejection

Parameter	Recipients	
	without rejection	with rejection
Age (years)	48.4 ± 9.9	49 ± 10.4*
Male (n,%)	16 (64%)	27 (87%)
Pre-HT diagnosis (n,%)	DCM – 17 (68%) CHD – 5 (20%) Other – 3 (12%)	DCM – 18 (58%) CHD – 10 (32%) Other – 3 (10%)
Tacrolimus concentration (ng/ml)	9.9 [6.1; 12]	8.1 [6.7; 10.7]*

Note. * – p > 0,05, compared with recipients without rejection.

Table 4

Comparative analysis of microRNA expression in heart transplant recipients with and without rejection

miRNA, $\log_2(2^{-\Delta Ct})$	Recipients		Significance, p
	without rejection	with rejection	
miRNA-101	–7.13 [–8.09; –4.87]	–8.21 [–9.60; –6.22]	**0.04**
miRNA-142	–7.02 [–8.04; –5.73]	–6.36 [–8.72; –5.11]	0.51
miRNA-27	–4.65 [–5.32; –1.85]	–5.19 [–6.78; –3.86]	**0.03**
miRNA-339	–10.04 [–11.2; –8.22]	–10.26 [–11.62; –9.2]	0.81
miRNA-424	–7.04 [–7.47; –5.79]	–7.05 [–8.27; –5.92]	0.98

CONCLUSION

Expression levels of miRNA-101, miRNA-27, miRNA-339 and miRNA-424 in patients with end-stage chronic heart failure – potential heart recipients – are higher than in healthy persons. A year or more following transplantation, the expression levels of miRNA-101, miRNA-142 and miRNA-339 in heart recipients do not differ from that in healthy persons. This may reflect normalization of adaptation processes in the graft.

Differences in the expression levels of miRNA-101 and miRNA-27 were found in recipients with acute graft rejection compared with recipients without it. This may be potentially important for assessing the risk of graft rejection and the possibility of minimizing immunosuppressive therapy. To assess the diagnostic effectiveness of miRNAs, further studies on the expression profile of these biomarkers in heart recipients are needed.

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THE NECESSITY OF VOIDING CYSTOURETHROGRAM FOR THE EVALUATION OF RECIPIENT CANDIDATES IN ADULT RENAL TRANSPLANTATION

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Objective. While international guidelines necessitate Voiding Cystourethrogram (VCUG) for pediatric patients, it is unnecessary for the evaluation of adult patients without urological disorders as renal transplant candidates. The objective of this study was to evaluate the results of adult candidates who underwent VCUG before transplantation and to demonstrate the necessity for this imaging. **Methods.** A retrospective study of the data of 1265 adult candidates who underwent VCUG before transplantation at our center, was undertaken. VUR, the presence of Post-voiding residual urine (PVR) (>100 ml), Low bladder capacity (LBC) (<100 ml), and urethral pathologies were evaluated with VCUG. **Results.** The mean age was 42.3 ± 1.3. The mean dialysis period was 27.8 ± 4.2 months. According to the VCUG results, 19.2% of the patients had pathological findings. On the other hand, the rate of urological disorders was only 5.1%, according to end-stage renal disease (ESRD) etiologies. VCUG outcomes indicated bilateral high-grade reflux in native kidneys in 4.4% (n = 56) of the candidates, unilateral high-grade reflux in 4.1% (n = 52), bilateral low grade reflux in 2.1% (n = 26), unilateral low-grade reflux in 2.4% (n = 30), and reflux in rejected transplanted kidney in 2.3% (n = 29). In addition, significant LBC was noted in 4.8% (n = 61), significant PVR in 1.1% (n = 14), and urethral stricture in 0.5% (n = 6) of the candidates. **Conclusion.** VCUG should be considered as a part of routine evaluation in adult renal transplant recipient candidates as well as in pediatric candidates, even if their ESRD etiologies are not due to urological disorders.

Keywords: renal transplantation; transplant candidates; vesicoureteral reflux; voiding cystourethrogram.

ABBREVATIONS

BOO – Bladder Outlet Obstruction
BPH – Benign Prostate Hyperplasia
ESRD – End Stage Renal Disease
LBC – Low Bladder Capacity
PVR – Post-Void Residuel Urine Volume
TURP – Transurethral Resection of the Prostate
VCUG – Voiding Cystouretrogram
VUR – Vesico Ureteral Reflux
UTI – Urinary Tract Infection

INTRODUCTION

Today, renal transplantation is considered the optimal treatment for patients with end-stage renal disease (ESRD), providing favorable physical, socioeconomic, and psychological results [1]. Accurate assessment of recipient candidates is of paramount importance to the success of renal transplantation. Therefore, candidates are subjected to many examinations and evaluations before transplantation. As a part of urologic examinations, voiding cystourethrogram (VCUG) is an important radiologic test that provides information about vesicoureteral reflux (VUR) and anatomical evaluation of the lower urinary system, as well as information about bladder functions. But the use of VCUG in the assessment of candidates is controversial. Urologic disorders account for up to 60% of the etiology of ESRD in pediatric patients [2]. In adult patients, urologic disorders account for 1.4% to 5% of the factors playing a role in the etiology of ESRD [3]. While international guidelines require VCUG in the evaluation of pediatric kidney transplant candidates, VCUG is not necessary in adult patients except for those with a medical history of genitourinary abnormalities [4, 5]. The objective of this study is to evaluate the results of adult candidates who underwent VCUG before transplant and to demonstrate the necessity of this imaging.

MATERIALS & METHODS

In this retrospective study, patients who underwent renal transplantation in Medical Park Hospital Complex Antalya Turkey between November 2008 and October 2011 were evaluated. Pediatric renal transplant patients under 17 were excluded. Of the 1,441 adult patients who underwent kidney transplant, the completed data of 1,265 patients who had undergone VCUG before transplant were studied. The study analyzed patients' demographic data; the urologic etiology of ESRD, such

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as the presence of obstructive or reflux uropathy; dialysis duration; and VCUG findings. VUR, the presence of post-void residual urine (PVR), low bladder capacity (LBC), and urethral pathologies were assessed with VCUG. Grading of vesicoureteral reflux is based on the International Reflux Study Committee Scale [6].

In VUR, Grades I and II were considered low-grade reflux; Grades III, IV, and V were considered high-grade reflux. Values less than 100 cc were considered significant in LBC, and values greater than 100 cc were considered significant in PVR.

The study was approved by the local ethics committee and written informed consent was received from all candidates. All statistical analyses were performed using the SPSS statistical software (SPSS for Windows version 16.0 SPSS Inc., Chicago IL, USA). Continuous variables were presented as mean ± standard deviation.

RESULTS

The data of 1,265 patients who underwent VCUG were examined. The mean age was 42.3 ± 1.3 years and male-to-female ratio was 2.2. The mean dialysis duration was 27.8 ± 4.2 months. According to VCUG outcomes, 19.2% (n = 243) of patients had abnormal pathological findings in the pre-transplantation assessment. Urologic disorders were involved in the etiology of ESRD in 5.1% (n = 65) of the patients. VCUG outcomes indicated bilateral high-grade reflux in native kidneys in 4.4% (n = 56) of the patients, unilateral high-grade reflux in 4.1% (n = 52), bilateral low grade reflux in 2.1% (n = 26), unilateral low-grade reflux in 2.4% (n = 30), and reflux in rejected transplanted kidney in 2.3% (n = 29). In addition, significant LBC was noted in 4.8% (n = 61), significant PVR in 1.1% (n = 14), and urethral stricture in 0.5% (n = 6) of the candidates (Table).

Table

Distribution of abnormal findings determined by VCUG

Type of abnormality	Prevalence
Bilateral High-Grade VUR in Native Kidneys	4.4%
Unilateral High-Grade VUR	4.1%
Bilateral Low-Grade VUR	2.1%
Unilateral Low-Grade VUR	2.4%
VUR in rejected TX Kidney	2.3%
Low Bladder Capacity	4.8%
Post-Void Residual Urine	1.1%
Urethral Stricture	0.5%

DISCUSSION

Voiding cystourethrogram (VCUG), also called a micturating cystourethrography, is a fluoroscopic study of the lower urinary tract in which contrast is introduced into the bladder via a catheter. The most important feature distinguishing VCUG from the other imaging modalities is that it is an interactive test. VCUG shows not only the anatomic plane of the low urinary tract and the presence of VUR, but like a urodynamic test, it can demonstrate filling phase and capacity of the bladder as well as the voiding phase and voiding ability of the patient. Thus, it can show whether urodynamic investigation is necessary. VCUG is unnecessary in adult renal transplant candidates unless there is a genitourinary abnormality present, as shown by two studies in the 333 and 517 case series conducted more than two decades ago [7, 8]. Among these studies, Glazier et al. found significant abnormalities of only 2.5% in VCUG performed at pretransplant evaluation [8]. Our study, however, showed abnormalities of 19.2% with VCUG. Similarly, Kabler et al. found 25% abnormalities with VCUG in candidates before transplantation [9]. Ultimately, VCUG is a diagnostic imaging method and 19.2% and 25% abnormalities found in our study and in the mentioned study are significant in showing significance of a test. A solution to these disparities needs to be found to minimize complications that may develop after transplantation.

Low bladder capacity is directly correlated with dialysis duration. It is the most important symptom of a dysfunctioning bladder, which develops due to decreased diuresis. Men are at greater risk of bladder dysfunctions [10]. Storage dysfunction becomes prominent one year after dialysis, especially in male patients over 50 [11]. Fortunately, reduction in bladder capacity is reversible and transplant generally allows restoration of bladder maximal output, normal bladder capacity, and compliance. Studies have shown that low bladder capacity dramatically increases within one year after transplantation [12, 13]. In patients with low bladder capacity, maximum detrusor pressure may require VUR in the transplanted kidney. The risk for VUR in the transplanted kidney within one year was significantly higher in patients with a low bladder capacity than those with a normal capacity [14]. Independent of immunological status, graft survival is significantly lower in patients with a severe bladder dysfunction such as a decreased maximal detrusor capacity less than 100 mL compared to other patients [15].

In conclusion, it may be risky to merely wait for patients with a low bladder capacity to return to normal. Many patients can be managed with anticholinergic drugs and intermittent self-catheterization, but some patients may require bladder augmentation or urinary diversion before transplant. It should be kept in mind that morbidity is lower and quality of life is better with intermittent self-catheterization compared with surgical approaches [15]. In a study by Song et al. evaluating pre-transplant VCUG outcomes, the rate of less than 100 mL bladder capacity was 14.1% in patients with a mean dialysis duration of 59 months [16] our study found the prevalence of low bladder capacity at 4.8% with a mean dialysis duration of 27 months. We think that the most important factor causing this difference between

results of the studies was dialysis duration. It would be reasonable to perform VCUG to determine bladder capacity well before transplant, especially in patients with a dialysis duration longer than one year.

VUR is a prevalent disease, and VCUG is the gold standard in its diagnosis. The prevalence of VUR in the population is higher in younger patients and decreases with age, although VUR is seen in 5% of sexually active women in the non-transplanted population [17]. It is difficult to determine the exact incidence of vesicoureteral reflux in patients undergoing renal transplantation because VCUG is not performed routinely in clinical practice. It should be remembered that these patients may be asymptomatic for VUR. VUR symptoms such as frequently repeating urinary tract infection (UTI) may be suppressed, especially inanuric and oliguric patients who receive dialysis. Immunosuppressive treatments with increased diuresis after transplant may cause persistent infection of the native kidneys due to VUR, leading to morbid or mortal outcomes [9]. International guidelines recommend that consideration be given to the need for native nephrectomy pretransplant or perioperatively in selected patients to reduce the risk of post-transplant complications [18]. In our study, VUR was found in 15% of the candidates, according to VCUG results. Similarly, Song et al. found VUR of 17.5% [16]. Similar results from both studies indicate that, unlike low bladder capacity, the prevalence of VUR is not correlated with dialysis duration in these patients.

PVR is an important pathological finding that can be detected with VCUG. Unlike low bladder capacity, high PVR is not expected during post-transplant recovery. Complications that may develop due to high levels of PVR will persist after transplantation, which may directly affect graft survival. Dysfunction of the bladder that causes long-term high intravesical pressure and significant post-voiding residual volume can cause VUR in the transplanted kidney and can pose a risk to graft function. Consequently, it is advantageous to diagnose these disorders as soon as possible, ideally prior to transplantation [19]. Studies conducted in the non-transplanted patients have reported a direct correlation between high PVR and UTI [20, 21]. UTI is the most common cause of post-transplant mortality and morbidity, affecting the duration of hospitalization [22]. Therefore, it is important to seek and plan treatment for the underlying causes of PVR. It should be remembered that UTI, which results most commonly from neurogenic bladder or bladder outlet obstruction (BOO), may be suppressed in anuric and oliguric patients who receive dialysis. Initiation of intermittent self-catheterization in a neurogenic bladder immediately after transplant is a highly efficient way to protect the transplanted kidney against infection and VUR. Another cause of PVR is BOO, which develops due to benign prostate hyperplasia (BPH). It should be kept in mind that male kidney transplant candidates over 50 are at a higher risk for BOO [23]. However, surgical treatment before transplantation is not recommended in patients undergoing dialysis. Because anuria will persist following the transurethral resection of prostate (TURP), these patients will be at high risk for development of bladder neck contracture in the postoperative period [24]. In addition to the initiation of alpha blockers in patients with severe lower urinary tract symptoms, it was shown that TURP or transurethral incision of the prostate (TUIP) operations can be performed safely in the first month after transplantation [23].

Another remarkable finding of VCUG was a urethral stricture in 0.5% of the patients. Urethral stricture may be seen with LBC in patients who receive dialysis for a long time [25]. As in patients with BOO and ESRD, symptoms may also be masked in these patients; diagnosis can be established during VCUG. In patients considered to have urethral stricture as a result of strain or failure of urethral catheterization at the anterior or bulbar urethra during VCUG the stricture can be restored by performing an internal urethrotomy before transplantation. This will likely prevent a surprise for the transplant team.

VCUG is a minimally invasive imaging method. Complications that may occur with this method should be taken into account. It should be remembered that oliguric and anuric patients are more likely to develop UTI after catheterization of the bladder. A resulting infection can cause postponement of the kidney transplantation, which may result in serious stress to the candidate. There is no sufficient data in the literature regarding a UTI resulting from a VCUG performed in candidates before transplantation. In a study investigating VUR in non-transplanted pediatric patients, UTI was found in 16.9% of children one week after VCUG was performed [26]. Prophylactic antibiotics given before VCUG may reduce this rate to a value as low as 1.7% [27]. Therefore, prophylactic antibiotics should be considered in candidates before VCUG in cases of infection risk.

This study has some limitations. Follow-up outcomes of patients with positive VCUG findings were not mentioned in this study.

CONCLUSION

VCUG should be considered a part of routine evaluation in adult kidney recipient candidates even if the etiology of ESRD is not related to urologic disorders. Candidates who received dialysis for longer than one year and male candidates over 50 should be given a routine assessment with VCUG. Pathologies that may directly affect graft survival after transplant can be diagnosed with VCUG.

The study were approved by Medical Park Hospital Local Ethics Committee (Protocol number: 2019/013).

The authors declare no conflict of interest.

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AGE-RELATED FEATURES OF THE PATTERN OF LYMPHOCYTE SUBPOPULATIONS AND FUNCTIONAL ACTIVITY OF PERIPHERAL BLOOD MONONUCLEAR CELLS IN PATIENTS WITH CHRONIC KIDNEY DISEASE BEFORE AND AFTER TRANSPLANTATION

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Objective: to analyze the features of the pattern of lymphocyte subpopulations and the functional activity of peripheral blood mononuclear cells in older adult patients with chronic kidney disease. **Materials and methods.** The study featured 21 patients with chronic kidney disease (CKD), over 55 years of age, who underwent kidney transplantation (KT) from unrelated suboptimal donors. The average age was 61.4 ± 4.5 years (55 to 69). Comorbidity was assessed using the CIRS-G scale; the average number of points was 13.6 ± 5.09. The control group consisted of 21 volunteers, aged 55–70, without acute inflammatory diseases and signs of chronic kidney disease (CKD). The average age was 61.1 ± 4.4 years, the average CIRS-G score was 12.11 ± 6.04. In all patients, the pattern of lymphocyte subpopulations of peripheral blood was evaluated by flow cytometry. Vital computer laser cytomorphometry was used to assess the functional state of peripheral blood mononuclear cells. The Functional Activities Index (FAI) was evaluated to indirectly assess the degree of functional activity of cells. **Results.** In CKD patients before KT, there was a decrease in the proportion of CD4 cells ($p = 0.009$), an increase in the proportion of CD8 cells ($p = 0.02$), a decrease in the CD4/CD8 ratio ($p = 0.017$), an increase in the proportion of natural killers ($p = 0.025$) compared with healthy volunteers. Moreover, a decrease in the total proportion of CD3 cells, an increase in HLA-DR expression on CD3 cells, and an increase in the proportion of B cells were statistically insignificant: $p = 0.137$, $p = 0.072$ and $p = 0.135$, respectively. On the fifth day after KT, the proportion of CD3 cells increased ($p = 0.017$) mainly due to an increase in the proportion of CD4 cells ($p = 0.002$) compared to the pre-KT index. The proportion of natural killers ($p = 0.002$) and HLA-DR expression on CD3 cells ($p < 0.0001$) also increased. An increase in the proportion of CD8 cells and in the CD4/CD8 ratio, and a decrease in the proportion of B cells were statistically insignificant: $p = 0.439$, $p = 0.277$, and $p = 0.236$, respectively. A decrease in FAI was noted in patients with CKD before KT in comparison with healthy volunteers ($p = 0.0138$). After ATP, this indicator significantly increased compared to the pre-KT value ($p < 0.0001$) and exceeded the FAI value in healthy volunteers ($p < 0.0001$). In healthy volunteers, there was no significant correlation between the functional activity of peripheral blood mononuclear cells and age ($r = –0.263$ [95% CI –0.6236; 0.1907], $p = 0.264$, $r^2 = 0.069$). At the same time, significant negative correlation between FAI and age was noted in CKD patients: $r = –0.52$ [95% CI –0.7771; –0.1135], $p = 0.0157$, $r^2 = 0.27$ before KT; $r = –0.418$ [95% CI –0.7559; –0.06256], $p = 0.0272$, $r^2 = 0.175$ after KT. **Conclusion.** Older adult CKD patients before and after KT were likely to have significant changes in the morphofunctional state of peripheral blood mononuclear cells and pattern of lymphocyte subpopulations. Moreover, the severity of changes in the functional state of these cells had a strong correlation with age, which was not observed in the group of healthy volunteers. This should be considered when choosing immunosuppressive therapy in older kidney transplant recipients.

Keywords: chronic kidney disease, kidney transplantation, lymphocyte subpopulations, cell functional activity.

INTRODUCTION

The number of patients with stage 5 chronic kidney disease (CKD) is steadily growing, as evidenced by reports from broad professional communities both in Russia and abroad. This is caused by a significant increase in the availability of renal replacement therapy, the increasing prevalence of diseases manifested by renal failure, as well as significant improvements in quality of the current renal replacement therapy which increases the life expectancy in this category of patients [1–3].

Among all methods of renal replacement therapy, optimal is cadaver kidney allotransplantation (CKAT) which provides the best medical and social rehabilitation, life quality and life expectancy in patients [1–3]. The growing shortage of donor organs has led to revision of the principles of their distribution. At present, fun-

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damental is their quality [4]. The main way to increase the number of donor organs, including kidneys, is to use organs obtained from suboptimal (marginal) donors, e.g. advanced criteria donors (unstable hemodynamics, diabetes mellitus, hypertension, trauma, age-related donors, etc.) [5–7].

An important problem is kidney transplantation to "age-related" recipients. Compared to young, the recipients of kidney allografts of older age groups are featured by the presence of an unfavorable premorbid background associated with the high rate of concomitant chronic diseases (arterial hypertension, coronary heart disease, heart failure, diabetes mellitus, chronic anemia, etc.) and a high polymorbidity index. Clinical manifestations and complications of chronic kidney disease are disguised as a therapeutic symptom complex that determines diseases of the internal organs. This makes transplantation particularly difficult in this group and stresses the importance of choosing the optimal immunosuppressive therapy.

The practical implementation of the "old for old" principle results in this category of patients having better chances of receiving an organ from a suboptimal donor than younger patients. Besides, the features of immunity in elderly patients should be considered when choosing an adequate immunosuppressive therapy [8–11]. There is no doubt that it is the choice of the optimal immunosuppressive therapy that plays the leading role in the long duration of the graft functioning and the recipient's life. In this regard, the study of various aspects of the age-related features of immune homeostasis in patients who are awaiting kidney transplantation seems an important and urgent issue.

Purpose: to analyze the features of the pattern of lymphocyte subpopulations and the functional activity of peripheral blood mononuclear cells in older adult patients with chronic kidney disease.

MATERIALS AND METHODS

The study included 21 patients with chronic kidney disease (CKD) over 55 years of age who underwent kidney transplantation (KT) in 2010–2018. The average age was 61.4 ± 4.5 years (55–69). CKD causes were glomerulonephritis, 23.8% (5 patients); pyelonephritis, 23.8% (5 patients); hypertension, 23.8% (5 patients); diabetes mellitus. 19% (4 patients); polycystic kidney disease, 9.5% (2 patients). Comorbidity was assessed by the Cumulative illness Rating Scale for Geriatrics (CIRS-G) scale; the average score was 13.6 ± 5.09.

All patients received renal replacement therapy, 19 patients – hemodialysis, 2 patients – peritoneal dialysis.

In compliance with the "old for old" principle [13, 14], all donor kidneys were obtained from suboptimal donors: "aged" and "asystolic" donors: categories IV (cardiac arrest after diagnosis of brain death, "controlled donor") or V (sudden cardiac arrest in patients in the intensive care unit, "uncontrolled donor") by the modified Maastricht classification of non-heart beating donors [15]. The preservation period averaged 13.2 ± 3.4 hours. In the vast majority of cases, only kidneys were taken from donors.

Selection of the donor-recipient pair was carried out considering the blood group and human leukocyte antigens of A, B and DRB1 loci. Depending on the number and combination of mismatches by the loci, the compatibility index was calculated [16]: the median was 6 (interquartile range from 6 to 7).

In all recipients, it was the first transplantation; there were no pre-existing anti-HLA antibodies. Antibody screening was performed by multiplex technology on the Luminex platform with LIFECODES Lifescreen Deluxe reagents (Immucor). Transplantation was performed only with negative cross-sectional tests (complement-dependent microlymphocytotoxic test).

All patients received standard induction therapy using anti-CD25 antibodies and methylprednisolone. A three-component immunosuppression protocol was used, tacrolimus (concentration control and subsequent dose adjustment), mycophenolates and prednisone in standard dosages [17].

All patients underwent standard postoperative clinical laboratory, radiological, and ultrasound examinations.

The control group included volunteers (N = 21) 55–70 years of age without acute inflammatory diseases and signs of renal failure. The average age in the control group was 61.1 ± 4.4, the average number of CIRS-G scores was 12.11 ± 6.04.

Immunophenotypy was performed by flow cytometry with the FACSCalibur apparatus (Becton Dickinson, USA). Multiparameter flow cytofluorimetric subpopulation analysis was made.

To assess the functional state of peripheral blood mononuclear cells, we used the method of vital computer laser cytomorphometry with the "Cytoscan" (MGIREA, Russia) laser phase-interference microscope. The method allows for capturing a phase-interference image of living cells and evaluate the level of anisotropy of nuclear chromatin, thus providing an indirect judgement of the degree of functional activity assessed by the functional activity index (FAI) of the core of the separated peripheral blood mononuclear cells.

The protocol of the present study was approved by the local ethics committee (protocol No. 4 of April 6, 2010) and by the decision of the academic council of the Vladimirsky Moscow Regional Research and Clinical Institute, Moscow (protocol No. 4 of April 19, 2010). All participants signed an informed consent.

STATISTICAL ANALYSIS

The distribution law compliance of the samples was checked by Shapiro–Wilk test. The variables with normal

distribution are presented as mean ± SD; those with the distribution differing from normal, as well as ordinal variables, are represented as the median and interquartile range: median (1st quartile; 3rd quartile).

To analyze the relationship between quantitative characteristics, Pearson correlation coefficient was used, the correlation coefficient (r), its 95% confidence interval (95% CI), and the determination coefficient (r^2) were calculated.

To analyze several samplings with a normal distribution, the analysis of variance with Post-hoc Tukey was implemented. The calculations were made with GraphPad Prism 8.0 (GraphPad Software, USA). 2-tailed significance was assessed. $p < 0.05$ was considered statistically significant.

RESULTS

To analyze the features of the cellular immunity unit activation at kidney transplantation, the differences in lymphocyte subpopulations in healthy volunteers and patients with CKD 5D stage receiving dialysis treatment before and after transplantation were analyzed (Fig. 1).

Comparing cells subpopulations in CKD 5D patients on dialysis with those of healthy volunteers, the variability is seen to significantly increase witnessing high heterogeneity of the dialysis patient population. With a statistically insignificant tendency to decrease in the proportion of CD3 cells ($p = 0.137$), the proportion of CD4 cells ($p = 0.009$) is significantly reduced and the proportion of CD8 cells ($p = 0.02$) increases. As a result of such multidirectional dynamics, the immunoregulatory index decreased (CD4/CD8 ratio), $p = 0.017$.

Besides, in dialysis patients, the number of natural killers increases ($p = 0.025$) and there is a tendency of the B cells ratio to slightly increase ($p = 0.135$), as well as increase in HLA-DR expression by CD3 cells ($p = 0.072$).

After KT, on day 5 after surgery, the proportion of CD3 cells statistically significantly increases ($p = 0.017$) mainly due to an increase in the proportion of CD4 cells ($p = 0.002$) with a slight increase in the proportion of CD8 cells ($p = 0.439$). As a result, the immunoregulatory index increased slightly ($p = 0.236$). The content of natural killers also increased ($p = 0.002$), and the proportion of B cells decreased slightly ($p = 0.277$). HLA-DR expression on CD3 cells increased significantly ($p < 0.0001$).

In CKD 5D patients, there is a decrease in the functional activity of peripheral blood mononuclear cells in comparison with healthy volunteers, which is reflected in a statistically significant ($p = 0.0138$) decrease in FAI. After KT, this indicator significantly increases compared with the indicator before KT ($p < 0.0001$) and exceeds the FAI value in healthy volunteers ($p < 0.0001$) (Fig. 2).

We noted interesting age-related features (Fig. 3).

In healthy volunteers, the functional activity of peripheral blood mononuclear cells gradually decreases

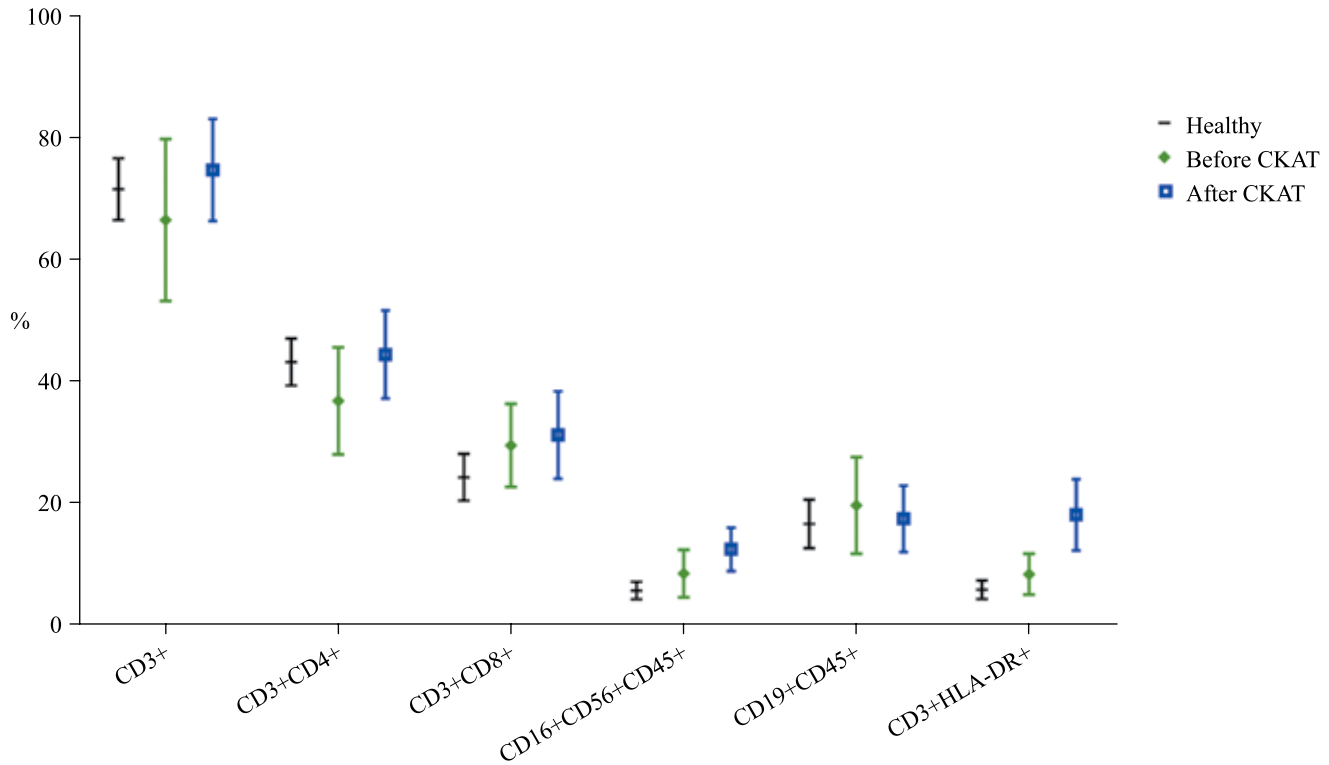

Fig. 1. Lymphocyte subsets, HLA-DR+ cell and natural killer cells fractions in healthy volunteers, CKD 5D patients before and after kidney transplantation

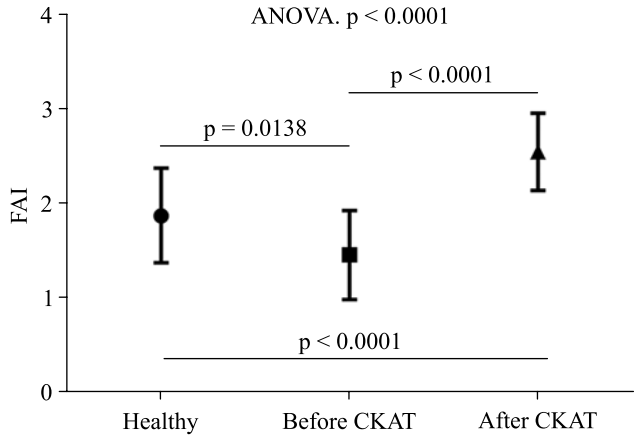

Fig. 2. Functional activity value of peripheral blood mononuclear cells in healthy volunteers, patients with CKD 5D before and after kidney transplantation

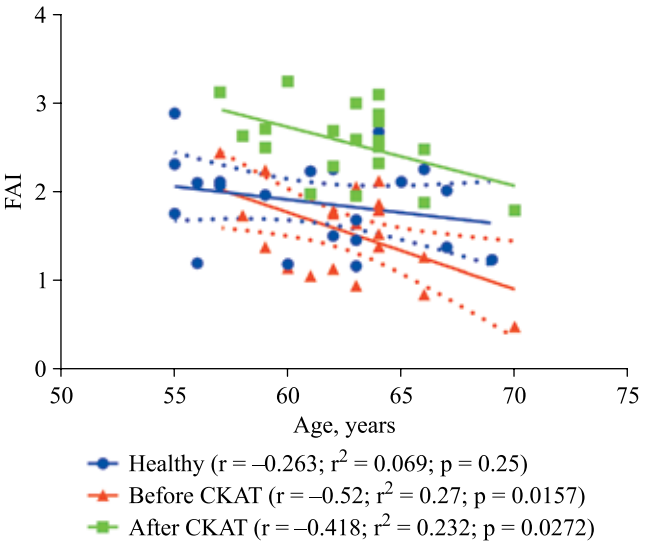

Fig. 3. Correlation of age and functional activity value of peripheral blood mononuclear cells in healthy volunteers, patients with CKD stage 5D before and after kidney transplantation

with age. Nevertheless, this relationship was weak (r = –0.263 [95% CI –0.6236; 0.1907], r^2 = 0.069) and did not reach the required level of statistical significance (p = 0.264). At the same time, in CKD5D patients, this dependence was of the same orientation, but was much stronger: (r = –0.52 [95% CI –0.7771; –0.1135], r^2 = 0.27) and was statistically significant (p = 0.0157). In patients after kidney transplantation, the average level of PFA was higher than before transplantation (p < 0.0001, Fig. 2), but the relationship with age was similar: r = –0.418 [95% CI –0.7559; –0.06256], r^2 = 0.175 and was statistically significant (p = 0.0272).

DISCUSSION

Age-related features of immune reactions have been known for long. The recipients of older age groups are featured by changes in the ratio of various subpopulations of lymphocytes, as well as in metabolism and functional potential of these cells [18–21]. We focused on the peculiarities of the subpopulation composition of lymphocytes and the morphofunctional state of peripheral blood mononuclear cells in CKD5D patients, namely in patients of an older age group before and after kidney transplantation.

The patients with chronic kidney disease are known to develop chronic inflammation combined with persistent native and adaptive immunity dysfunction [22]. The clinical manifestation of this fact, as well as its indirect confirmation, is an increased risk of infectious complications [23, 24] and malignant neoplasms [23, 25], as well as a decrease in the efficiency of vaccination [26, 27], as evidenced by the results of large studies.

We found that in stage 5 CKD patients, there is a decrease in the proportion of CD4 cells and an increase in the proportion of CD8 T cells, while their ratio is significantly reduced. Besides, there is a slight decrease in the total proportion of CD3 cells (statistically insignificant in the present study) with a significant increase in the variability of this indicator, which indirectly points to the heterogeneity of the CKD patient population.

There is evidence that the lymphopenia severity is associated with several factorsб the severity of impaired renal function and oxidative stress, the level of urea, creatinine, phosphorus, diabetes mellitus, etc. [28–32]. The change in the subpopulation composition of lymphocytes, as well as a decrease in their total number, is tied to an increased tendency towards apoptosis, a transformation of the cytokine profile, which is characteristic of patients with CKD [30, 33–35]. Besides, the dialysis procedure itself promotes apoptosis of T cells and reduces their proliferative ability [29, 31, 35].

Impaired T-cell regulation associated with progressive loss of renal function is often described as “premature aging of the immune system”, which is featured by a significant reduction in the native T-cell population and a relative increase in memory T-cells, a decrease in the ratio of CD4/CD8 T-cells, and an increased tendency to apoptosis, a change in the receptor repertoire of T cells and a reduction in telomere length. In patients with CKD of 25–45 years old, the indicators are comparable with those of healthy people of 60 to 80 [30, 36, 37].

The severity of changes in T-cell immunity is directly related to the results of kidney transplantation. An increase in the waiting time for kidney transplantation on dialysis promotes the accumulation of alloreactive T cells and is accompanied by an increased risk of acute transplant rejection [38, 39] and significantly worsens the results of transplantation [40–42]. Despite the fact that the characteristics of T-cell immunity influence the results of kidney transplantation [32, 43, 44], currently this knowledge does not allow modifying the existing

clinical practice by personalizing immunosuppressive therapy, which emphasizes the relevance of research in this direction. The fact that the features of violations of T-cell immunity (in the context of the "aging" of the immune system) can affect not only the immediate ones (since the existing disorders form a kind of pre-transplant background), but also the long-term results of kidney transplantation, according to research results, which have been shown that these disorders persist even after successful transplantation [32, 37].

Natural killer cells (NK) are one of the specialized subpopulations of lymphocytes that play an important role in antiviral and antitumor immunity, as well as in the regulation of homeostasis and inflammatory processes in tissues [45, 46]. In our study, the proportion of these cells in CKD patients was significantly greater than in healthy volunteers. This indicates the activation of native immunity, which may be of a compensatory-adaptive nature against the background of dysfunction of adaptive T-cell immunity in CKD [47]. On the other hand, an increase in the proportion of NK in patients with CKD can be explained by the active participation of these cells in the progression of CKD (regardless of the underlying etiology of kidney disease) [48–51].

The proportion of B cells in healthy individuals and CKD patients did not differ statistically significantly. Nevertheless, in the latter it was slightly higher, as was the heterogeneity of this indicator. There are also several non-mutually exclusive explanations for this. B cells play an important role in the pathogenesis of many autoimmune renal lesions [52–54]. In our study, a significant proportion of patients with formal CKD were glomerulonephritis and hypertension (10 of 21). Nevertheless, it should be borne in mind that histological verification of the diagnosis (causes of CKD) is extremely rare, which does not exclude the possibility of another reason. The fact that the structure of the causes of CKD in our country, according to the data of the All-Russian Register [2], is significantly different from other large registers [1, 3] also indirectly testifies to this. In addition, for example, we did not take into account the form of glomerulonephritis (which can be attributed to the limitation of the study). Another explanation (arising from the first) may be the use of various drugs to treat the underlying disease (causes of CKD). For example, rituximab (an anti-CD20 monoclonal antibody) is widely used to treat many autoimmune diseases: ANCA-associated vasculitis, membranous nephropathy, lupus nephritis, mixed cryoglobulinemia, nephrotic syndrome with minimal changes, focal segmental glomerulosclerosis etc. [47, 55]. It should be noted that there is no consensus on the polarity of the change in the proportion of B cells in CKD patients [56, 57].

In the present study, CKD patients had an increased level of HLA-DR expression compared with healthy individuals. This may be due to chronic inflammation of a low degree of activity characteristic of CKD patients [22, 58, 59].

In kidney transplant recipients, on day 5 after transplantation, an increase in the proportion of CD3 cells, CD4 cells, NK, as well as the expression of HLA-DR was noted. This can be considered, on the one hand, as a nonspecific response to surgery [60–63]; on the other hand, a synchronous increase in the proportion of CD4 cells and NK may be a specific reaction to allotransplantation [64–69].

If we do not take into account the problem of presensitized patients, then severe humoral reactions to the graft, the clinical manifestation of which is the rejection reaction, usually refer to the late postoperative period and occur with the active participation of adaptive immunity. We deliberately sampled patients without preexisting anti-HLA antibodies. In the pathogenesis of ischemia-reperfusion syndrome, the main role is played by nonspecific reactions of native immunity with activation, mainly of the cellular link. In this regard, we evaluated the morphological and functional parameters of the cellular immunity unit (as the main effector unit) in renal allograft recipients.

After transplantation, an increase in the activity of peripheral blood mononuclear cells occurs (even compared with healthy volunteers). This is quite expected and can be explained by both nonspecific and specific mechanisms. At the same time, in CKD 5patients, both before and after transplantation, a statistically significant relationship between PFA and age was revealed, which was not observed in the group of healthy volunteers. This fits quite well into the modern concept of violations of the morphofunctional state of the cells of the immune system formed against the background of CKD. However, the revealed dependence testifies in favor of the fact that the age of the recipient is an important factor that can influence the choice of immunosuppressive therapy regimen. This becomes even more relevant in view of the fact that elderly patients are characterized by a significant change in the metabolism of immunosuppressive drugs and, accordingly, their concentration in the blood (in particular, calcineurin inhibitors), which makes it even more difficult to achieve a shaky balance between insufficient and excessive immunosuppression [8–11, 70, 71].

CONCLUSION

Older CKD patients before and after CKAT are susceptible to a significant change in the morphofunctional state of peripheral blood mononuclear cells and the subpopulation of lymphocytes. Moreover, the severity of changes in the functional state of these cells has a strong relationship with age, which is not observed in the group of healthy volunteers. This should be considered

when choosing immunosuppressive therapy in kidney transplant recipients of an older age group.

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The authors declare no conflict of interest.

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SCREENING OF CADAVER CORNEA DONOR FOR INFECTIONS IN THE EYE BANK OF THE FYODOROV EYE MICROSURGERY FEDERAL STATE INSTITUTION

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Objective: to analyze negative laboratory results of cadaver cornea donor screening during preparation of corneas for transplantation according to data from the internal registry of donors of the eye bank (EB) of the Fyodorov Eye Microsurgery Federal State Institution and the European Eye Bank Association (EEBA) from 2011 through 2015. **Materials and methods.** Data analysis was carried out using the internal registry of EB donors and the EEBA annual directories. The analyzed data included the number of eyeballs obtained, the frequency of incomplete tests (hemolysis for EB) and positive serological results for human immunodeficiency virus (HIV-1 and HIV-2), viral hepatitis B, viral hepatitis C and syphilis. **Results.** In just 5 years, the EB received 3,479 eyeballs. After hemolysis of donor blood samples, 13.9% (n = 486) of corneas were excluded from the EB. EEBA recorded fewer inconclusive tests during the same period. After hemolysis and positive serological tests, 19.4% (n = 676) of corneas were excluded from the EB. Overall, the number of positive serological tests in EBs was far higher than in the EEBA data. Frequency of positive HIV tests (HIV-1 and HIV-2) and syphilis in EB showed low variability annually, while incidence of hepatitis B increased in 2015. For the analyzed period, positive serology for hepatitis C was found to be prevalent among EB donors. Mixed infections were quite often recorded in blood samples. **Conclusion.** Based on analysis conducted, positive serology and hemolysis were the main contraindications and led to exclusion of 33.3% (n = 1162) of cadaver donor corneas received in EB. Frequency of positive serological tests for indicated infections in EB was higher than in the EEBA data, with significant predomination of hepatitis C.

Keywords: corneal transplantation, eye bank, serological tests, human immunodeficiency virus, hepatitis B virus, hepatitis C virus, syphilis.

INTRODUCTION

The eye bank (EB) of the Acad. S.N. Fyodorov Eye Microsurgery Federal State Institution was founded in 1988 to improve the practice of cornea donorship in the USSR (the Order of Academician S.N. Fedorov, General Director, No. 150 of 21.11.1988). Since 1995, the EB has possessed full membership in the European Eye Bank Association (EEBA) [1], taking an active part in the Association activities including its annual conferences. Currently, the EB prepares donor tissue for all types of keratoplasty and plays a key role in cornea transplantation in Russia.

Infection screening as an integral part of the EB work aims at preventing infectionstransmission from donor to recipient through a cornea transplant. The practice reports two cases of transmission of hepatitis B virus through a cornea transplant from a donor positive for the "Australian antigen" are known in [2]. At the same time, cases of transmission of human immunodeficiency virus (HIV) type 1 and 2, viral hepatitis C, and the syphilis causative agent through cornea donor tissue have not yet been reported [3, 4]. Taking into account the extreme importance of screening for infections at preparing and preserving a cornea transplant, the EB examines blood samples from each donor enrolled for these infections according to the licensing regulations.

In accordance with the Algorithm for preparing human cadaver cornea for transplantation [5], expert pathologists in thanatology departments perform initial screening of post-mortem donors. Donors bearing traces of injections on the body, tattoos, patients with tuberculosis, those died from burn injuries, as well as in cases of prescription of biological death of over 24 h and over 70 of age, are excluded. The EB receives the donor material in the form of eyeballs after enucleation by the thanatological service. Mandatory is the serology of blood samples from each donor. These biological materials are delivered in a sealed container and stored at +40 °C until the results of serological tests are ready. All blood samples received from cadaver donors are serologically tested for HIV (type 1 and 2), viral hepatitis B, viral hepatitis C, and syphilis. Thuis is done in the clinical

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laboratory of the Fyodorov Eye Microsurgery licensed to work with group III–IV group infectious pathogens. Samples with hemolysis and positive results for indicated infections are disposed of in accordance with the Russian SanPiN (Sanitary Rules and Regulations). Eyeballs from uninfected donors proceed to the next step to perform morphological and functional screening. Next, the transplantability index (morphological equivalent of a function) is determined, the corneoscleral disk is cut out and hypothermically preserved in the Borzenka–Moroz medium for subsequent clinical use [6].

In European Eye Bank Association, there is no single standard for harvesting donor corneas, though screening for infections is also performed for HIV (types 1 and 2), viral hepatitis B and C, and syphilis. Earlier, S.A. Borzenko has analyzed the infection of corneal cadaver donors for the period of 1996–2005 [6]. However, a comparative analysis of the incidence of the indicated infections for this period of time was not performed.

MATERIALS AND METHODS

Data analysis was carried out using the internal registry of EB donors and the EEBA annual directories. The analyzed data included the number of eyeballs obtained, the frequency of incomplete tests (hemolysis for EB) and positive serological results for human immunodeficiency virus (HIV-1 and HIV-2), viral hepatitis B, viral hepatitis C and syphilis.

RESULTS

From January 2011 to December 2015, the EB received 349 cadaver eyeballs with the median of 566 per year ranging from 556 (the 1st quartile) to 692 (the 3rd quartile).

Every year, hemolysis was the cause of utilization of 13.9% of the corneas by median (13.8–14.0%, quartiles 1–3) received in the EB. In the EEBA system, the term "Serology test inconclusive or impossible" is used, and according to the associationб the number of such tests was 2.0% (median) per year (1.2–2.1%, quartiles 1–3) (Fig. 1, b).

Positive HIV (types 1 and 2) tests in the analysis of blood samples in the EB were recorded in 1.2% of cases (median) with low variability over years: 1.1–1.7% (quartiles 1–3). In the EEBA, 3 times less positive HIV (type 1 and 2)tests were noted, with the median of 0.4% (Fig. 1, a, b).

Positive tests for viral hepatitis B in donor blood samples in the EB were recorded in 5.3% of cases (median) with a sharp, over twofold increase to 10.7% in 2015. In the same period, the EEBA recorded 2.9 times fewer positive results for viral hepatitis B (Fig. 1, a, b) with low annual variability.

Hepatitis C tests in the EB were positive in 12.1% (median) of donor blood samples with a peak of 14.5% in 2012, and a decline to 8.3% in 2014. In the same period, in the EEBA, the frequency of positive tests hepatitis C was 13.4 times lower than the median compared with the EB data. There was weak variability over the years and a general downward trend for this indicator. (Fig. 1, a, b).

Positive serological tests for syphilis in blood samples of donors in both the EB and the EEBA were weakly variable, though differing 7.7 times in median (Fig. 1, a, b).

DISCUSSION

In the EB, screening for infections of cornea cadaver donors helps prevent recipients from becoming HIV-, viral hepatitis B and C-, and syphilis-infected. The functioning medical and technological system of the EB provides for compulsory recording of the results of each serological examination performed at preparing a preserved cornea transplant. For thirty years of continuous daily work, the unique statistical data has been collected that allows to track the frequency of occurrence of the identified infections and hemolysis over a given period of time.

In 2011–2015, the EB hemolysis of blood samples of corpse donors resulted in rejection of 486 corneas, or 13.9% of the incoming material. In this period, the EB HIV seropositive tests (types 1 and 2), hepatitis B, hepatitis C and syphilis led to rejection of 676 corneas or 19.4% of the incoming material. In total, from 2011 to 2015, 33.3% of donors (n = 1162) has been excluded due to infections detected in the blood samples or due to hemolysis.

According to the results of the analysis, it was found that such infections as HIV, viral hepatitis B, viral hepatitis C, and syphilis occur, respectively, 3; 2.9; 13.4; 7.7 times more often according to the EB internal register compared to the EEBA data. This stress the fact such studies should take into account medical, social and other characteristics of the population [7]. Guided by the official data of the Federal State Statistics Service and the Ministry of Health [8], for 2011–2015, we have not found any correlation between the rate of positive serological tests and the incidence rate of the indicated infections (Fig. 2). In this regard, further study of the results of screening for infections of cornea donors in the eye banks of the Russian Federation is necessary.

CONCLUSION

In the course of the present study, it was found that from January 2011 to December 2015. the eye bank (EB) of the Fyodorov Eye Microsurgery Federal State Institution has received 3,479 eyeballs. All blood samples from cadaver donors were subjected to serology in the clinical laboratory of ща the Institute for HIV (types 1 and 2), viral hepatitis B and C, and syphilis. Serology showed 19.4% of seropositive donors, while hemolysis was detected in 13.9% of cases. Comparing these results with the data of the European Association of Eye Banks for the same period, seroprevalence of such infections as

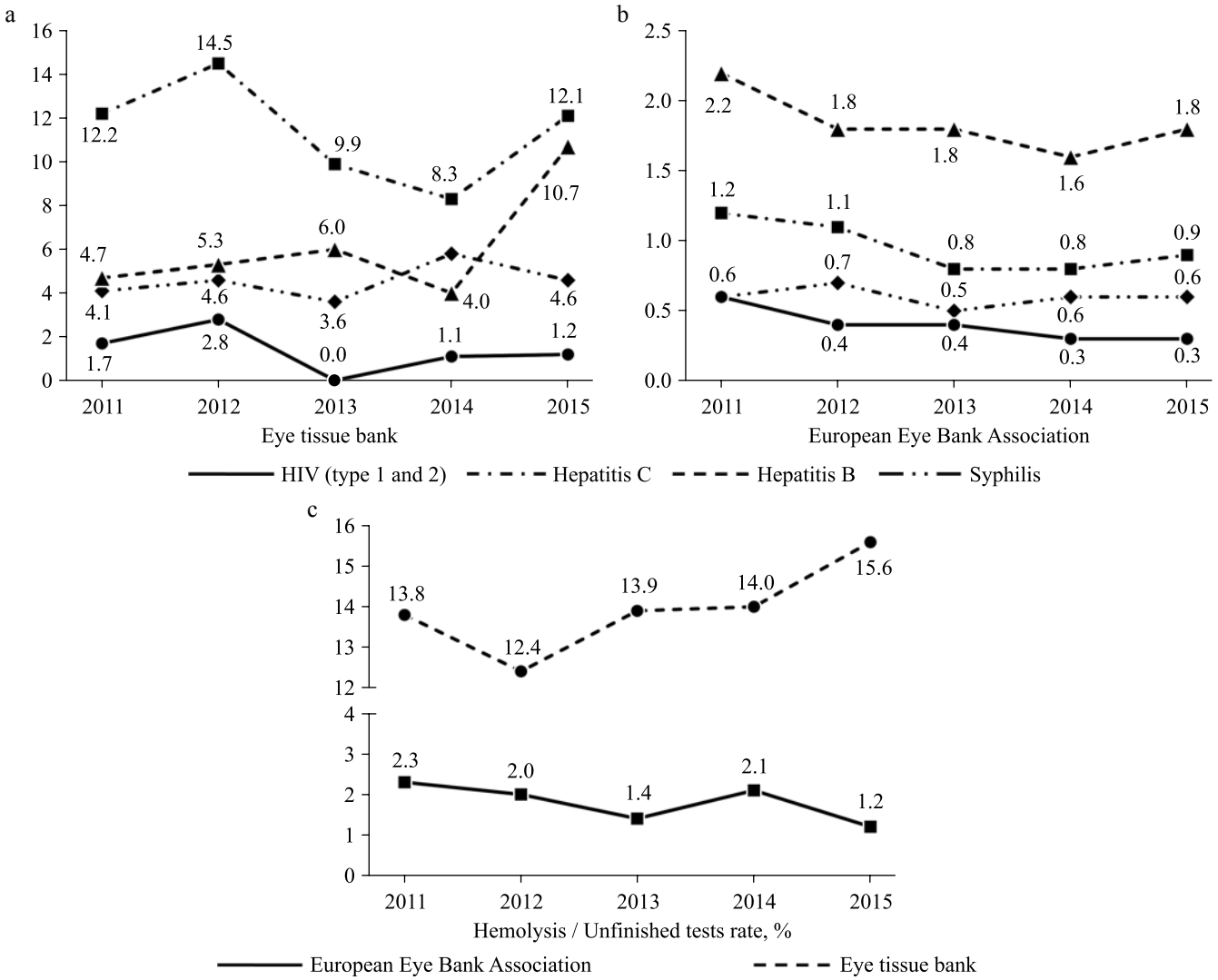

Fig. 1. Corneal donor screening results from 2011 through 2015. Rates of positive serology tests for human immunodeficiency virus (type 1 and 2), Hepatitis B and C viruses, and Syphilis in Eye tissue bank Eye Microsurgery (a) and European Eye Bank Association (b); rates of hemolysis in donor blood samples in in Eye tissue bank Eye Microsurgery and European Eye Bank Association (c)

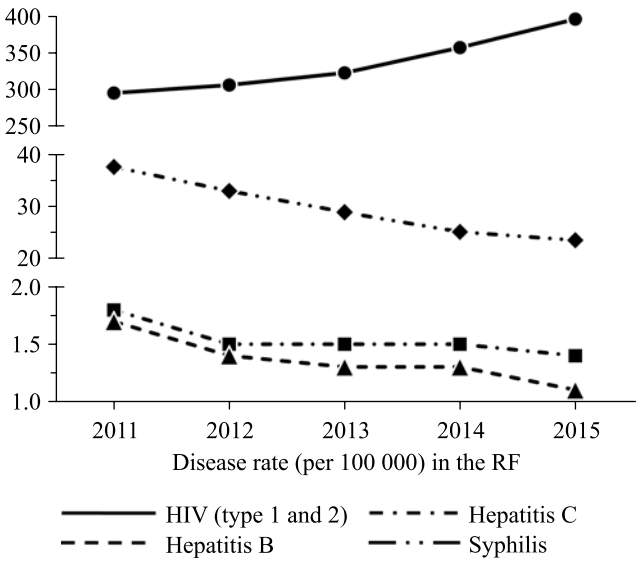

Fig. 2. Statistical data on the disease rate for human immunodeficiency virus (type 1 and 2), Hepatitis B and C viruses, and Syphilis in the Russian Federation according to the report of the Ministry of Health and Federal State Statistic

HIV, viral hepatitis B, viral hepatitis C, and syphilis was found to be higher in the EB donors. At the same time, no correlation was found between the rate of positive serology and the above infections rates in the population of the Russian Federation in 2011–2015.

Thus, screening for infections of cornea cadaver donors can prevent recipients to be infected with HIV, viral hepatitis B and C, syphilis, and develop prognostically significant algorithms for the prevention of recipient contamination with indicated hemo-transmissible infections in the eye banks in Russia.

The authors declare no conflict of interest.

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BOTKIN HOSPITAL TRANSPLANT PROGRAM: 100 SOLID ORGAN TRANSPLANTATIONS

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Objective: to evaluate the first results of the Botkin Hospital transplant program. **Materials and methods.** From June 2018 to October 2019, 100 solid organ transplants were performed at the Botkin City Clinical Hospital. Out of the 100 transplantations, 72 were kidney transplants (average age of recipients was 45.65 ± 11.35 years, HLA match averaged 2.09 ± 1.03) and 28 were liver transplants (average age of recipients was 50.14 ± 7.62 years, average MELD was 17.78 ± 3.28 (14–34). **Results.** After transplantation, there was no 30-day mortality. Postoperative complications following kidney transplantation were established in 11 patients (15.2%). In 3 patients (4.3%) – suppuration of postoperative wound, in 2 patients (2.8%) – hematomas in the area of the postoperative suture during hemodialysis, in 5 patients (6.9%) – retroperitoneal lymphocele, in 1 patient (1.4%) – urosepsis. There were 4 cases (5.5%) of acute rejection, 3 cases (4.2%) of humoral rejection, and 1 case (1.3%) of cellular rejection. Early postoperative complications following liver transplantation were detected in 2 patients (7.2%). In one patient – hematoma under the right lobe of the liver on the 1st day after surgery, the diaphragm was the source of bleeding, in one patient – ascites leakage through postoperative sutures, which required relaparotomy. In 2 patients (7.2%), postoperative complications were found in the separated postoperative period. In one case, of choledochocholedochal anastomotic stricture – stricture stenting was performed with coated nitinol stent. In another case, acute adhesive intestinal obstruction, which required laparotomy, adhesiolysis. **Conclusion.** Implementation of the transplantation program in multidisciplinary hospitals can boost transplant care in a district and improve the treatment results of patients with terminal organ damage.

Keywords: kidney transplantation, liver transplantation, Botkin Hospital.

INTRODUCTION

Transplant care in Moscow has been a hot topic in modern medicine [1, 2]. As of December 31, 2018, 1529 people were on the waiting list for cadaveric kidney transplants in Moscow, while 208 people were in the liver waitlist [3]. Over half of patients awaiting transplantation are of working age. In Moscow, 10 billion roubles per year is spent on renal replacement therapy with hemodialysis. More than 3 billion roubles per year are spent on treatment of patients with incurable liver diseases [4]. This identifies the medical, financial and social problems of treating these patients [5].

The solution to these problems is to further develop transplant technology [6, 7]. So, over the past 4–5 years, the number of effective donors in Moscow has been steadily growing, donated organs have been increasing in number [3], thus increasing the number of transplant surgeries.

MATERIALS AND METHODS

Botkin City Clinical Hospital (Botkin Hospital) is Russia's largest multidisciplinary hospital with more than 1800 beds. The hospital provides medical care in all areas of treatment. It has a full range of laboratory and instrumental diagnostics. Implementation of a transplant program at the hospital included three stages, which took place simultaneously throughout the year.

The first stage was about legal issues. The first step in this stage involved the inclusion (via Order No. 307n/4 of the Ministry of Health of the Russian Federation, dated June 4th, 2015) of Botkin Hospital in the registry of health care institutions engaged in harvesting, preparation, and transplantation of human organs and (or) tissues. Further, by Order No. 404n/1 of the Ministry of Health of the Russian Federation dated July 11th, 2017, Botkin Hospital was included in the registry of health care institutions of the constituent entities of the Russian Federation performing transplantation of human organs and (or) tissues. In the final step in this first stage, Botkin Hospital obtained Roszdravnadzor license (dated November 29, 2017) for medical activities on provision of specialized assistance in transplantation of organs and (or) tissues.

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The second stage was a scientific one. It involved training of specialists from Botkin Hospital under the auspices of the Shumakov National Medical Research Center of Transplantology and Artificial Organs and the Sklifosovsky Research Institute of Emergency Care. In total, 26 employees were trained under the transplant program. Among them were 6 surgeons, 4 urologists, 1 gastroenterologist, 2 anesthetists, 5 resuscitators, 2 laboratory diagnostics doctors, 4 nephrologists, and 2 surgical nurses. Participants in the "Botkin Hospital Organ and Tissue Transplantation" program actively participated in liver and kidney transplantation surgeries at the Sklifosovsky Research Institute of Emergency Care and Shumakov National Medical Research Center of Transplantology and Artificial Organs. They also sharpened their skills in the cadaver-class pathology department of Botkin Hospital.

The third stage is the clinical one, which consisted of development of regulations for provision of high-tech medical care in kidney and liver transplantation. At this stage, waiting lists for cadaveric kidney and liver were formed.

KIDNEY TRANSPLANTATION

The first cadaveric kidney transplant at Botkin Hospital was performed on June 7, 2018. The patient had chronic glomerulonephritis. Over the 16 months that the Botkin Hospital Transplant Program existed, 72 kidney transplants were performed.

For all kidney donors, death was ascertained based on neurological criteria (brain death). Acute cerebrovascular accident (stroke) was the cause of death in 62.3% of donors. Mean age of donors was 46.44 ± 9.7 (22–65) years. In all donors, blood electrolyte values were within or slightly higher than normal (K^+ – 4.06 ± 0.64 (3–7.0) mmol/L, Na^+ – 142.14 ± 11.24 (127–165) mmol/l). Average creatinine level was 93.58 ± 27.69 (44–180) mmol/L, and urea – 6.06 ± 2.43 (2.0–10.9) mmol/L.

The mean age of recipients was 45.65 ± 11.35 (20–70) years. The HLA match averaged 2.09 ± 1.03 (1–5). However, it should be noted that all pairs had a match for at least one DR antigen. There were 5 (6.9%) pre-dialysis patients; 17 (23.6%) recipients also needed an emergency hemodialysis session before surgery.

Average cold ischemia time was 585.41 ± 191.93 (133–1188) min, average blood loss was 104.29 ± 52.54 (30–300) mL, average operation time was 250.72 ± 40.6 (160–370) min. A ureteral stent was installed in all kidney transplant cases. It was removed at the end of week 3 post-transplantation. Average bed day in ICU after kidney transplantation was 1.55 ± 0.85 (1–4) bed days. Total number of postoperative bed days following kidney transplantation was 17.08 ± 6.18 (12–33). Postoperative complications were observed in 11 patients (15.2%), postoperative wound suppuration in 3 patients (4.3%), hematomas in the site of postoperative suture during hemodialysis in 2 patients (2.8%), retroperitoneal lymphocele in 5 patients (6.9%), urosepsis – in 1 patient (1.4%). There were 4 cases (5.5%) of acute rejection, 3 cases (4.2%) of humoral rejection, and 1 case (1.3%) of cellular rejection. There was no 30-day mortality. After kidney transplantation in all cases, a 4-component immunosuppressive regimen was used (monoclonal antibodies, glucocorticosteroids, calcineurin inhibitors, inosine monophosphate dehydrogenase inhibitors).

LIVER TRANSPLANTATION

The first cadaveric liver transplant took place on July 8, 2018. The patient had primary biliary cholangitis.

Over the 15 months that the Botkin Hospital Transplant Program existed, 28 liver transplants were performed.

Mean age of donors was 41.96 ± 10.46 (22–56) years. Average AST (57.97 ± 58.84 (7–208) IU/L) and ALT (42.85 ± 38.59 (5–184) IU/L) slightly exceeded the norm (40 IU/L). Average bilirubin level was within normal limits – 11.89 ± 9, 14 (3.7–43) IU/L.

Mean age of recipients was 50.14 ± 7.62 (34–66) years. Average MELD score was 17.78 ± 3.28 (14–34).

All liver transplants were performed using the standard piggyback technique. Choledochocholedochal anastomosis was formed in 25 cases, continuous absorbable monofilament thread 6-0 – in 3 cases. Average cold ischemia time was 396.82 ± 68.88 (290–590) min, and average warm ischemia time was 37.78 ± 8.88 (20–60) min. Average blood loss was 1322.22 ± 752.6 (200–3000) mL, average operation time was 531.1 ± 59.2 (430–720) min. Average bed day in ICU after liver transplantation was 3.04 ± 1.47 (1–8) bed days. Total number of postoperative bed days following liver transplantation was 16.33 ± 5.14 (11–37). There was no 30-day mortality. Early postoperative complications were observed in 2 patients (7.2%). In one patient, a hematoma under the right liver lobe was detected on day 1 after surgery. Diaphragm was the bleeding source. In one patient, ascites leaks through postoperative sutures was found. They required relaparotomy, drainage of the abdominal cavity. In 2 patients (7.2%), postoperative complications were revealed in the long-term postoperative period. In one case, choledochocholedochal anastomotic stricture – coated nitinol stent was used for the stricture. In another case, there was acute adhesive intestinal obstruction, requiring laparotomy, adhesiolysis. There were no histologically documented acute rejection reactions. There was no mortality. In liver transplantation for autoimmune diseases, a 4-component regimen was used (n = 4). A 3-component or 2-component regimen (n = 24) was used (monoclonal antibodies, calcineurin inhibitors, inosine monophosphate dehydrogenase inhibitors) for other conditions.

The third area of the Botkin Hospital Transplant Program was corneal transplantation. To date, ophthal-

mologists from Botkin Hospital have performed 160 operations at the hospital.

The fourth area of the program was autologous hematopoietic stem cell transplantation. The hematological clinic of Botkin Hospital performed 23 surgeries.

DISCUSSION

Increasing the availability of transplant care in the Russian Federation is an important medical, financial and social challenge. High-tech medical care via transplantation needs to be developed in leading regional medical institutions that have modern facilities and highly skilled medical personnel.

At the time the Transplant Program was launched, Botkin Hospital met all modern standards for transplant centers. The hospital has a nephrology center, a urology clinic, a liver & pancreas surgery department, a hepatology department, an anesthesiology and resuscitation center, an extracorporeal detoxification department, an ophthalmology clinic, and a hematology clinic. This made it possible for the hospital to – within a short time, with the involvement of its own staff, and with minimal financial costs – launch a transplantation program in four directions, and within 1 year and 4 months perform 100 solid organ transplantations with good immediate outcomes, which are comparable to the outcomes of other transplant centers.

CONCLUSION

Introduction of a transplant program in multidisciplinary hospitals would increase the volume of transplantological care in a particular region and improve treatment outcomes for patients with end organ damage.

The authors declare no conflict of interest.

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AMBIGUOUS RESULTS OF BALLOON ANGIOPLASTY FOR CENTRAL VEIN STENOSIS IN HEMODIALYSIS PATIENTS WITH NATIVE ARTERIOVENOUS FISTULA

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Objective: to conduct comprehensive comparative analysis of the patency rate of native arteriovenous fistula (AVF) for central vein stenosis (CVS) after endovascular balloon angioplasty and palliative surgery. **Materials and methods.** The retrospective study included 80 patients with confirmed central vein stenosis: subclavian, brachiocephalic veins, inferior vena cava, or multiple lesions. The experimental group included 39 patients who underwent percutaneous balloon angioplasty. The control group included 41 patients who, for various reasons, did not do balloon angioplasty, but underwent palliative interventions: thrombectomy, proximalization of arteriovenous anastomosis, AVF blood flow-reducing surgical procedures. **Results.** Primary patency (time interval between the first intervention for CVS and the second intervention) in the experimental group was 61.5% [95% CI 44.5; 74.7] and 15.4% [95% CI 6.2; 28.3] at 6 and 12 months, respectively. In the control group, it was 39% [95% CI 24.3; 53.4] and 0% respectively. Hazard ratio (HR) 0.5337 [95% CI 0.3381; 0.8427], log-rank test p = 0.0011. No differences in functional primary patency (time interval between the start of using AVF and the first intervention for CVS) were found: 89.7% [95% CI 74.9; 96] and 30.8% [95% CI 17.3; 45.4] at 1 year and 3 years, respectively, in the experimental group, and 80.5% [95% CI 64.8; 89.7] and 24.4% [95% CI 12.7; 38.2] in the control group. There were no differences between the groups HR 0.7695 [95% CI 0.4952; 1.196], log-rank p = 0.2259. In the experimental group, strong negative correlation between primary patency and functional primary patency was detected: r = –0.627 [95% CI –0.787; –0.388], p < 0.0001. In the control group, no such correlation was found: r = 0.049 [95% CI –0.262; –0.351], p = 0.7599. Thus, the later CVS developed, the less effective balloon angioplasty was. Balloon angioplasty significantly increased duration of AVF use after first intervention for CVS (secondary patency): 84.6% [95% CI 68.9; 92.8], 66.7% [95% CI 49.6; 79.1] and 17.9% [95% CI 7.9; 31.3] at 6, 12 and 24 months, respectively in the experimental group. In the control group, it was 56.1% [95% CI 39.7; 69.6], 19.5% [95% CI 9.2; 32.7] and 0%. HR 0.4009 [95% CI 0.2481; 0.6477], log-rank p < 0.0001. Functional secondary patency (total duration of AVF use) was: 100%, 74.4% [95% CI 57.6; 85.3] and 12.8% [95% CI 4.7; 25.2] at 1, 3 and 5 years in the experimental group, and 95.1% [95% CI 81.9; 98.8], 36.6% [95% CI 22.3; 51] and 4.9% [95% CI 0.9; 14.5] in the control group. HR 0.5661 [95% CI 0.3598; 0.8906], log-rank p = 0.0067. **Conclusions.** 1. Central vein stenosis inevitably cuts vascular access from the ipsilateral side. 2. Balloon angioplasty allows to slightly prolong AVF use but it cannot radically change the long-term results of CVS treatment. 3. The outcome of balloon angioplasty greatly depends on the length of the period from the time the use of AVF started to the time CVS developed. 4. Multiple repeated balloon angioplasties are apparently justified in patients for whom creating a new vascular access might not be possible. 4. AVF volumetric blood flow velocity is an important factor determining the severity of CVS clinical manifestations and whether repeated surgical interventions are needed.

Keywords: central vein stenosis, arteriovenous fistula, hemodialysis vascular access, balloon angioplasty, percutaneous transluminal angioplasty, endovascular surgery.

INTRODUCTION

Vascular access is one of the key aspects in the survival of patients with chronic kidney disease (CKD) receiving treatment with long term hemodialysis (HD). From year to year, there has been a monotonous increase in the number of patients with stage 5 CKD. The rate of increase is gradually rising [1]. It is generally accepted that arteriovenous fistula (AVF) is the preferred vascular access for HD. Initiating HD with an AVF is associated with better survival compared to other types of vascular access [2–4].

Central vein stenosis (CVS) is one of the severe complications in patients on HD. It is known that CVS significantly increases the risk of loss of ipsilateral access. It

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also has many adverse manifestations from subclinical venous hypertension to superior vena cava syndrome [5]. CVS prevalence varies widely: from 2 to 40% [6–8]. An important aspect is the fact that CVS not only leads to loss of functioning vascular access, but also makes it impossible to create a new access from the ipsilateral side [6, 7]. This significantly reduces the "vascular resource" of formation of not only native AVF, but also of any type of vascular access.

Implantation of the central venous catheter (CVC) is a major etiological factor for CVS [9–11]. Despite the "fistula first" principle [12, 13], 21% of prevalent hemodialysis patients in the US were dialyzed with a CVC [14], in Europe – 28% [15], in Russia – 12% [16]. At the same time, the need for CVC is highest at the beginning of renal replacement therapy: 80% of HD patients in the USA [14] start dialyzing with a CVC, in Europe – 61% [15]. According to our data [17] (registry of CKD patients in the Moscow region) – 43% of patients in Moscow and Moscow Oblast start dialyzing with a CVC. Due to widespread high demand for CVCs, one cannot but hope for a spontaneous solution to the problem of CVS.

Endovascular surgery is one of the fastest growing areas of reconstructive vascular access surgery for HD. Despite the enthusiasm generated after the first reports on successful percutaneous balloon angioplasty in stenosis and recanalization of occluded central veins [18, 19], and the high probability of technical success (which, according to many authors, reaches 100% [20–22]), it was later established that long-term AVF patency is low [5, 23]. The revealed contradictions contributed to the rethinking of approaches to plastic surgery in central vein stenoses and occlusions, which, presumably, has not ended today. As experience was gained and clinical trials were completed, approaches to improving the outcomes of endovascular interventions were proposed. Thus, the use of stents [24] and stent grafts [25], drug-coated balloon catheters [26] and high-pressure balloon catheters [27] has been suggested. Such a variety of available methods of influencing the affected vein segment is compensated by the absence of specific indications that make it possible to choose an optimal method, which significantly compromises endovascular interventions. At the same time, percutaneous balloon angioplasty remains the most accessible method for restoring central vein patency in HD patients. We dedicated our research to analyzing the characteristics of the outcomes of primary angioplasty without stenting.

Objective: to conduct a comprehensive comparative analysis of the patency of native AVF for CVS after endovascular balloon angioplasty and palliative surgery.

MATERIALS AND METHODS

The study protocol was endorsed by a local ethics committee and approved by the academic council.

Patients

The retrospective study included 80 patients with confirmed CVS. The experimental group included 39 patients (48.75%) who underwent endovascular balloon angioplasty (BA). Thrombectomy was performed in case of thrombosis, which was supplemented by proximalization of arteriovenous anastomosis (AVA), if necessary. The control group included patients who, for various reasons, did not perform BA. Palliative surgeries were performed in this group: thrombectomy in case of thrombosis, which was supplemented by AVA proximalization if necessary, or by AVF blood flow-reducing surgical procedures by means of formation of a bandage from synthetic vascular prosthesis on the juxta-anastomotic segment of the "fistula" vein, if the indication for operation were clinical manifestations for venous hypertension in the limb. This group was composed of 41 patients (51.25%).

The main inclusion criteria were: above 18 years of age at the time of inclusion in the study, subclavian, jugular, and brachiocephalic vein stenosis, inferior vena cava, or their combination; AVF lasting for at least one month; availability of reliable information on anamnesis and catamnesis; loss of AVF. Patients who used stents, as well as patients who used synthetic vascular prostheses as vascular access, were excluded from the study (such observations were excluded from analysis).

In all patients, except for 5 (3 (7.7%) in the experimental group and 2 (4.9%) in the control group), the first AVF was created before the start of HD. However, a large proportion of patients initiated HD through CVC due to primary dysfunction: 25 (64.1%) in the experimental group and 28 (68.3%) in the control group. Prior to the first CVS intervention, patients underwent one to three interventions. Tunneled CVS was the preferred intervention. In both groups, the need for CVC was high. The main indicators in the groups are summarized in Table. To evaluate the comorbid background, the CIRS (Cumulative Illness Rating Scale) scale [28] in the Miller modification [29] was used as the most convenient for retrospective analysis in our center. When analyzing CKD causes, the "systemic processes" group included patients with vasculitis, myeloma, HIV infection, patients with kidney neoplasm (some of them are renoprival), patients who underwent chemotherapy, having a long history of drug addiction, etc.

In 23 patients of the experimental group and 26 patients of the control group, isolated subclavian, brachiocephalic or superior vena cava stenosis was revealed. In 16 patients of the experimental group and 15 patients in the control group, stenosis of one of these veins was combined with jugular vein stenosis. Central vein stenosis was confirmed by angiography or ultrasound. Moreover, in patients from the control group, stenosis in some cases

Table

Characteristics of the groups

	Experimental group (n = 39)	Control group (n = 41)	Significance of difference
Age (years)	45 [39.25; 50], 23 to 59[1]	47 [41; 55], 26 to 71[1]	p = 0.3999
Gender (M/F)	43.6%/56.4% (17/22)	41.5%/58.5% (17/24)	p = 0.8475
Comorbidity, CIRS scores	14.5 [12; 19.75], 7 to 26[1]	18 [12; 23], 7 to 29[1]	p = 0.0894
Cause of CKD			
Polycystic kidney disease	25.6% (10)	22% (9)	p = 0.993
Pyelonephritis	12.8% (5)	12.2% (5)	
Glomerulonephritis	15.4% (6)	17.1% (7)	
Diabetes	28.2% (11)	31.7% (13)	
System processes	17.9% (7)	17.1% (7)	
Time interval between AVF formation and its use (months)	4 [3; 4.6], 0.7 to 7[1]	3 [3; 4], 2 to 7[1]	p = 0.43
Time interval between the start of using AVF and the first intervention for CVS (months)	29 [18.5; 40.5], 6 to 54[1]	25 [16; 36], 4 to 51[1]	p = 0.2858
Need for reconstructive interventions before using AVF	3.704 [2.79; 4.82][2]	3.841 [2.917; 4.965][2]	0.964 [0.665; 1.396][3] p = 0.845
Need for reconstructive interventions from the start of using AVF to the first intervention for CVS	2.263 [1.478; 3.316][4]	2.241 [1.435; 3.334][4]	1.01 [0.577; 1.773][3] p = 0.9742
Number of CVCs before the first intervention for CVS	3 [2; 5], 0 to 7[1]	3 [2; 5], 0 to 8[1]	p = 0.763
Need for CVC before the first intervention for CVS	1.443 [1.205; 1.713][2]	1.298 [1.091; 1.532][2]	1.112 [0.875; 1.412][3] p = 0.3859
Number of catheters in relation to catheterization duration	4.72 [4.944; 5.605][5]	4.796 [4.032; 5.663][5]	0.984 [0.774; 1.25][3] p = 0.897
Average duration of use of one CVC (months)	1.4 [1.18; 1.8], 0.7 to 5.7[1]	1.3 [1.03; 1.98], 0.6 to 5.6[1]	p = 0.753
Stenosis localization:	(percentage of 39)	(percentage of 41)	
left subclavian vein	46.2% (18)	51.2% (21)	p = 0.9915
right subclavian vein	28.2% (11)	24.4% (10)	
left internal jugular vein	17.9% (7)	14.6% (6)	
right internal jugular vein	23.1% (9)	22% (9)	
left brachiocephalic vein	12.8% (5)	12.2% (5)	
right brachiocephalic vein	7.7% (3)	9.8% (4)	
superior vena cava	5.1% (2)	2.4% (1)	
Need for open reconstructive interventions after the first intervention for CVS	0.374 [0.24; 0.556][2]	2.451 [1.963; 3.023][2]	0.153 [0.095; 0.237][3] p < 0.0001
Need for balloon angioplasty	1.137 [0.89134 1.43][2]	–	–
General need for surgical interventions after the first intervention for CVS	1.511 [1.225; 1.843][2]	2.451 [1.963; 3.023][2]	0.617 [0.461; 0.825][3] p = 0.0011

Note. [1] Median, interquartile range. [2] Number of operations per 10 patient-months and 95% confidence interval. [3] Incidence rate ratio (intensity of occurrence of events) and 95% confidence interval. [4] Operations per 100 patient-months and 95% confidence interval. [5] Number of CVCs per 100 catheter days and 95% confidence interval.

was revealed during angiographic examination performed in connection with CVC implantation difficulties.

Estimated indicators

In accordance with the latest clinical recommendations [30], we evaluated the following indicators:

– Primary patency – the time interval between the first intervention for CVS and the first repeated surgical intervention (eventless survival of vascular access from the moment of first intervention for CVS);
– Assisted primary patency – the time interval between the first intervention for CVS and the first AVF thrombosis, including surgical open or endovascular interventions to maintain its function (non-occlusive vascular access survival from the moment of the first intervention for CVS);
– Secondary patency – the time interval between the first intervention for CVS and complete cessation of the use of AVF, including all surgical interventions.
– Functional primary patency – the time interval between the start of AVF and the first surgical intervention.

These indicators are similar to those described above with the only difference being that the start of measurement of the corresponding period was considered as the start of using AVF.

Statistical analysis methods

For quantitative features (e.g. age, average duration of CVC use), the median and interquartile range (first and third quartiles) were calculated. Comparisons were performed using the Mann–Whitney U test. For nominal values (e.g. gender, localization of stenosis), fractions were calculated. Comparisons were performed using the chi-square test.

Patency was assessed using the Kaplan–Meier estimate. The significance of differences was assessed using the Mantel–Cox Logrank test (long term) and Gehan–Breslow–Wilcoxon (short term). Point estimates and 95% confidence intervals (95% CI) were calculated. In addition, the survival median (and 95% CI) was calculated, i.e. point in time when the event did not occur in 50% of subjects. Relative risk of event was assessed using the hazard ratio – HR (log-rank).

In order to take the total number of events into account when doing risk assessment, the incidence rate ratio was determined, which is the intensity of the onset of events: the number of events for a standardized time interval (for example, the number of operations of 10 patient-months of follow-up). The ratio of the two incidence rate ratios (IRRs) was interpreted as relative risk.

Calculations were performed in GraphPad v.8 and OpenEpi v.3. A two-sided level of significance was evaluated. Values $p < 0.05$ were considered statistically significant.

RESULTS

Indicators of functional patency, as well as patency after the first intervention for CVS are shown in Figure.

We did not notice any significant differences in the functional primary patency (Fig., a) between the groups: 89.7% [95% CI 74.9; 96] and 30.8% [95% CI 17.3; 45.4] after one year and three years, respectively, in the experimental group, 80.5% [95% CI 64.8; 89.7] and 24.4% [95% CI 12.7; 38.2] – in the control group, HR 0.7695 [95% CI 0.4952; 1.196], $p = 0.2259$; median in the experimental group – 29 months [95% CI 22.9; 35.1], in the control group – 25 months [95% CI 19.8; 30.2].

Moreover, there was lesser need for second intervention after BA compared with palliative "open" interventions, as evidenced by primary patency rates (Fig., b): 61.5% [95% CI 44.5; 74.7] and 15.4% [95% CI 6.2; 28.3] after 6 and 12 months, respectively, in the experimental group, 39% [95% CI 24.3; 53.4] and 0% in the control group, HR 0.5337 [95% CI 0.3381; 0.8427], $p = 0.0011$; median in the experimental group – 8 months [95% CI 6; 10], in the control group – 6 months [95% CI 4.9; 7.1].

In the experimental group, a strong inverse correlation between the primary patency and functional primary patency was found: $r = –0.627$ [95% CI –0.787; –0.388], $r^2 = 0.393$, $p < 0.0001$. In the control group, there was no such correlation: $r = 0.049$ [95% CI –0.262; –0.351], $r^2 = 0.002$, $p = 0.7599$.

The total duration of use of AVF in the experimental group was significantly longer than in the control group, and as evidenced by functional secondary patency rates (Fig., c): 100%, 74.4% [95% CI 57.6; 85.3] and 12.8% [95% CI 4.7; 25.2] after one, three and five years in the experimental group, 95.1% [95% CI 81.9; 98.8], 36.6% [95% CI 22.3; 51] and 4.9% [95% CI 0.9; 14.5] in the control group, HR 0.5661 [95% CI 0.3598; 0.8906], $p = 0.0067$; median in the experimental group – 47 months [95% CI 40.9; 53.1], in the control group – 34 months [95% CI 29.8; 38.2].

At the same time, BA allowed to significantly increase AVF duration after the first operation for CVS, as evidenced by secondary patency rates (Fig., d): 84.6% [95% CI 68.9; 92.8], 66.7% [95% CI 49.6; 79.1] and 17.9% [95% CI 7.9; 31.3] after 6, 12 and 24 months, respectively, in the experimental group, 56.1% [95% CI 39.7; 69.6], 19.5% [95% CI 9.2; 32.7] and 0% in the control group, HR 0.4009 [95% CI 0.2481; 0.6477], $p < 0.0001$; median in the experimental group – 16 months [95% CI 12.5; 19.5], in the control group – 7 months [95% CI 4.9; 9.1].

The occlusion-free period from the moment the use of AVF was stated was longer in the experimental group than in the control group, as evidenced by the functional primary assisted patency rate (Fig., e): 100%, 61.5% [95% CI 44.5; 74.7] and 2.6% [95% CI 0.2; 11.5] after one, three and five years, respectively, in the experimen-

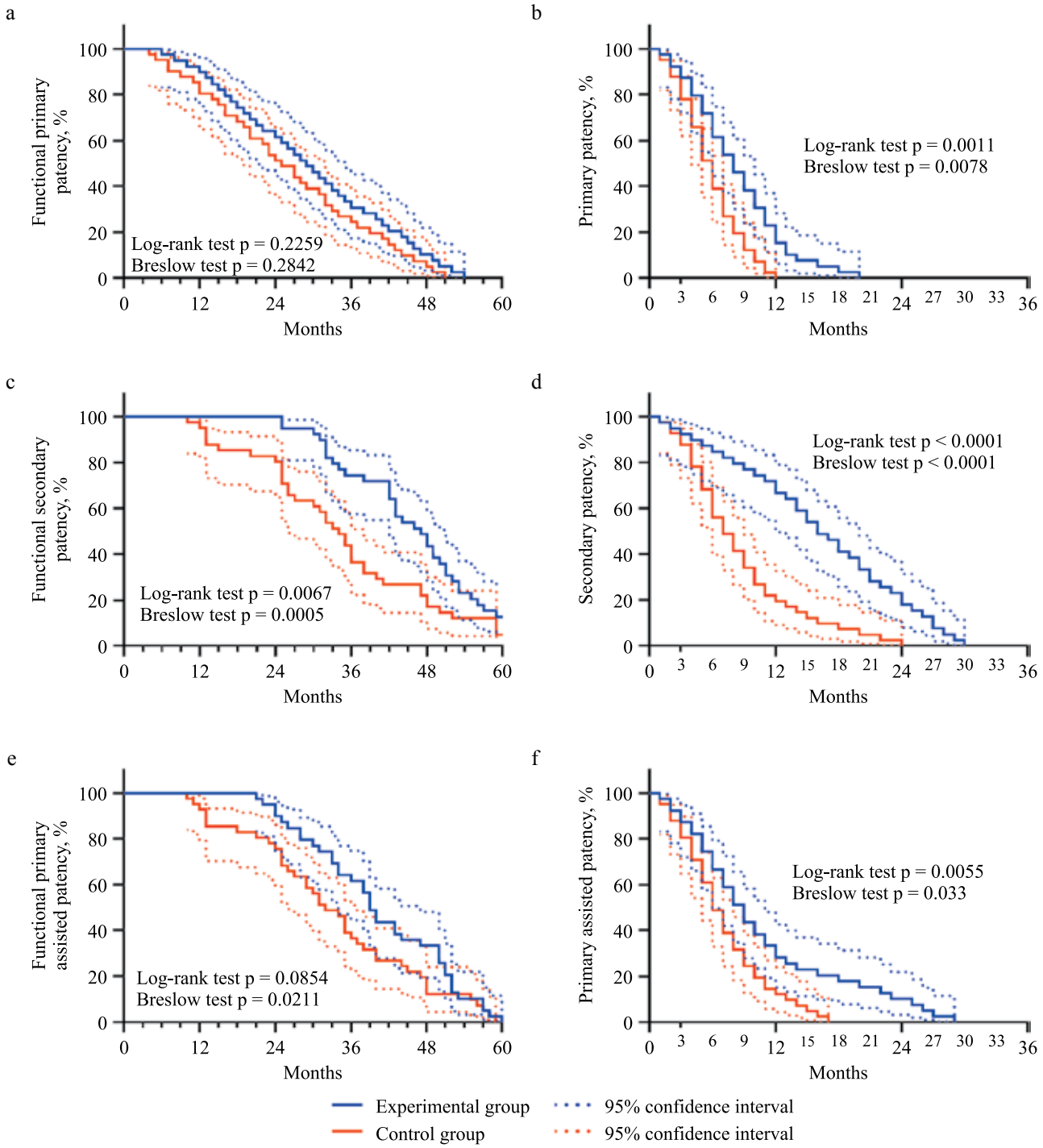

Fig. Functional patency rates – primary (a), secondary (c) and assisted primary (e); patency indicators after the first intervention for CVS – primary (b), secondary (d) and assisted primary (f). Red indicates a group of patients after balloon angioplasty, blue – after palliative "open" surgical interventions, the dots indicates 95% confidence intervals (Kaplan–Meier estimate)

tal group, 92.7% [95% CI 79; 97.6], 36.6% [95% CI 22.3; 51] and 0% in the control group, HR 0.7212 [95% CI 0.4633; 1.123], p = 0.1193; median in the experimental group – 39 months [95% CI 36.5; 41.5], in the control group – 32 months [95% CI 27.5; 36.5].

The occlusal period from the moment of the first surgical intervention was also significantly longer in the experimental group, as evidenced by the primary assisted patency rate (Fig., f): 66.7% [95% CI 49.6; 79.1], 28.2% [95% CI 15.3; 42.7] and 10.3% [95% CI 3.3; 22] after 6, 12 and 24 months, respectively, in the experimental group, 48.8% [95% CI 32.9; 62.9], 12.2% [95% CI 4.5; 24.1] and 0% in the control group, HR 0.5758 [95% CI 0.3664; 0.905], p = 0.0055; median in the experimental group – 9 months [95% CI 7; 11], in the control group – 7 months [95% CI 5.6; 8.4].

DISCUSSION

To increase the objectivity of the study, the sample was deliberately formed in such a way that all subjects had an outcome – AVF failure.

From Table it can be seen that both samples were obtained from the same set: we did not note any differences between the groups by main parameters. However, this population differs from the general HD patient population by distribution of CKD causes [17]. It is logical that the proportion of patients with CKD causes that predetermine the difficulties of providing constant vascular access (polycystic kidney disease, diabetes and systemic processes) were significantly higher. Although the first AVF creation in most patients was done before HD, a larger proportion of patients initiated HD via CKD. As a result, in 3–4 months before a stable vascular access was created and AVF was started, patients underwent an average of 3 reconstructive interventions performed in connection with primary failure: early thrombosis or delayed fistula vein maturation. Since the vast majority of these interventions consisted of AVA proximalization, by the time the use of AVA was started, most patients had AVF in the middle or upper third of the forearm (proximal AVF). We consider this an important factor in both the development of central venous stenosis and in its rapid clinical manifestation. It is known that the AVF high volumetric flow rate (which is characteristic of proximal AVF) leads to abnormal shear stress and turbulence. Non-physiological hemodynamics promotes endothelial dysfunction, activation of endotheliocytes and platelets, and neointimal hyperplasia. The vein walls thicken due to remodeling and fibrosis, [31–34]. At the same time, increased volumetric flow rate quickly leads to depletion of the functional-compensatory capabilities of the vein and its collaterals.

In the vast majority of cases, the main initiating factor for CVS is the use of CVC [9–11]. Indeed, the subjects had a high need for CVC (Table). Moreover, despite the fact that preference was given to permanent CVCs, the average duration (median) of using one was approximately 1.3–1.4 months. CVC dysfunctions or infectious complications required implantation of a new catheter. Despite the fact that this was not the immediate goal of our analysis, based on our own experience, we are inclined to conclude that the number of CVC implants is a more important risk factor for CVS development than catheterization duration. This has been confirmed by a number of studies [35–38]. Nevertheless, it should be noted that there is no consensus among researchers on this issue: some have the opposite opinion [36–39].

It is curious that in 4 patients (1 in the experimental group and 3 in the control group), CVCs were not used. It is known that idiopathic CVS is described, which nevertheless is extremely rare [40]. In this case, stenosis could occur in the area of confluence or branching, and in the area of twisted vein segments and anatomical bends, subject to increased pressure and sustained turbulent blood flow due to the presence of AVF from the ipsilateral side. [41, 42].

Symptoms of venous hypertension can occur in the absence of organic stenosis due to damaged vein walls. Functional stenosis may develop as a result of external compression of the vein by anatomical structures in the thoracic outlet [43–45]. This phenomenon is known as thoracic outlet syndrome [46]. Such observations in patients on hemodialysis are fairly well described [40, 47, 48]. In the most severe cases, Paget–Schroetter syndrome develops [49, 50]. The AVF high volumetric flow rate significantly promotes early clinical manifestation of vein compression (or organic stenosis) [40, 43, 44, 51–53].

External vein compression (as well as its physiological bends) against the background of direct arteriovenous discharge of a large volume of blood (as a result of AVF creation) can cause blood flow turbulence and promote neointimal hyperplasia and fibrosis [51, 54] of the vein wall. In this regard, chronic venous compression at the thoracic outlet level can be an important potential factor for CVS [55].

We noted a high incidence of subclavian vein stenosis, especially on the left. This agrees well with the data from other authors: the use of subclavian veins for catheterization (compared with the internal jugular veins) [7, 35, 37, 56, 57] and the use of left subclavian veins [7, 58] are associated with increased risk of stenosis. This can be explained by anatomical features: a more winding path to the right atrium, as well as a smaller vein diameter on the left [7, 10]. Nevertheless, such localization distribution of stenosis can distort reality, as localization of stenosis was one of the inclusion criteria. The study did not include patients with isolated jugular vein stenosis. This was done deliberately, since isolated jugular vein stenosis creates objective difficulties in CVC implantation, but affects AVF patency to a lesser extent.

A comparative analysis of treatment outcomes (comparing the patency rates with and functional patency rates) reveals some interesting nuances that allow to somewhat differently evaluate BA outcomes and supplement essentially the idea about optimal provision of stable vascular access for CVS patients.

Primary patency rates (Fig., b) are traditional: in the control group, one year after the first operation for CVS, all patients required another surgery. In the experimental group, by 15 months, 92% of patients needed another operation. Only a small group of patients required repeated surgical intervention at a later date. However, primary patency did not exceed 20 months.

Functional primary patency curves, on one hand, indicate that CVS manifestations requiring surgical treatment, can, with the same probability, occur at any time of its use. In both groups, the survival curves decrease almost linearly, which is an indirect sign that the intensity

of the onset of the event (the first repeated operation) is relatively constant. On the other hand, it is rather difficult to predict when the CVS clinical manifestation would occur. Obviously, this depends on many factors, among which we believe the main ones are the initial state of the patient's veins, AVF blood flow rate and a history of CVC use. The first surgeries for CVS in the experimental group were performed already after 6 months of AVF use, and in the control group – after 4. The combined influence of a number of factors contributed to the fact that clinically significant CVS had already formed in the patients within this period. In this regard, it is very important not only to identify significant risk factors for CVS (which is the subject of most of these studies), but also to assess with reasonable accuracy the unique impact of each of them on CVS *incidence* at different periods of treatment. It is likely that the impact of various factors on CVS risk will be different. For example, a patient who has successfully used one tunneled CVC for six months, and a patient who, for various reasons, underwent several catheterizations within one month, may have different risks of CVS. Results of such an analysis can serve as a reason for conducting, for example, angiography (an invasive and expensive method) for vascular access dysfunction in a patient at risk until clinically significant symptoms of venous hypertension appear.

We identified very important features when comparing primary patency with functional primary patency (Fig., a). These two indicators are inextricably linked. The endpoint for functional primary patency (first repeated operation after AVF formation) is the starting point for primary patency. In the experimental group, there was a strong inverse correlation between functional primary patency and primary patency: the later the first intervention for CVS was required, the earlier a repeated intervention would be required. This is understandable because hemodynamic disturbances against the background of a long-functioning AVF, on one hand, apparently lead to formation of the corresponding morphological substrate – change in the vein wall. On the other hand, a gradual increase of AVF blood flow (especially with proximal AVF) leads to manifestation of clinical signs of CVS. Since, as we have established, "late" stenoses are less treatable and after surgical resolution of CVS with BA, recurrence develops faster, the compensatory potential of venous collaterals does not have time to be fully achieved. No such dependence was revealed in the control group: duration of the first intervention for CVS to the second one did not depend on the length of time between AVF formation and CVS appearance. This can be explained by the fact that the essence of operations in the control group consisted of thrombectomy in case of thrombosis, which was supplemented by AVA proximalization if necessary or by reducing blood flow through AVF by forming a bandage from a synthetic vascular prosthesis on the juxtaanastomotic segment of the "fistula" vein, if the indication for the operation consisted of clinical manifestations of venous hypertension in the limb. Both the formation of a bandage and formation of a new AVA led to reduced blood flow through AVF. This, on one hand, indicates the important influence of this parameter on the clinical manifestations of CVS. On the other hand, the lack of significant correlation between functional primary patency and primary patency indicates that reducing AVF blood flow is an effective palliative operation for any CVS formation period (within our study), in contrast to the effectiveness of balloon angioplasty.

As follows from Fig., d, balloon angioplasty can significantly increase the secondary patency, i.e. the period between the first intervention for CVS and the complete loss of AVF function. Nevertheless, even in the experimental group, secondary patency does not exceed 30 months. In addition, differences between functional secondary patency curves are not so pronounced. In both groups, AVF function was completely lost 70 months after the start of AVF in the experimental group and 66 months in the control group. Despite the fact that differences between the groups were statistically significant (even in the long-term period, as evidenced by the P value for the log rank test), after 54 months, the confidence intervals cross the alternative survival curves. In other words, whenever CVS develops, its function will most likely be lost by 5 years after the start of AVF, regardless of the treatment method used. Given the fact that, according to the data in Table and Fig., a, the time interval between the start of using AVF and the first intervention for CVS (i.e., in fact, the duration for development of clinically significant CVS) did not differ between the groups. This can be explained by the fact that the effectiveness of balloon angioplasty decreases as the duration of AVF use increases. This is also confirmed by the presence of a significant inverse correlation between primary patency and functional primary patency. As a result, differences in secondary patency are partially offset.

At the same time, BA allowed to more than halve the risk of losing AVF function in the early stages of its use: in the control group, the first AVF was lost after 10 months, while in the experimental group – after 25 months (functional secondary patency – Fig., c).

Differences in primary and secondary AVF patency in the control group indicate that blood flow reduction is an effective palliative method for increasing AVF patency. However, there is no consensus on the optimal value of AVF volumetric blood flow rate. It must be remembered that significant decrease in this rate may increase the risk of AVF thrombosis [59–62]. In our study, whenever blood flow reduction was necessary, the target values were in the range of 1–1.5 liters per minute.

One of the important reasons for the higher functional secondary survival of AVF in patients from the experimental group is the fact that in the experimental

group, "open" surgical interventions only supplemented endovascular interventions if necessary, while in the control group, "open" interventions were the only option for surgical interventions. Moreover, since AVA proximalization was often required, it is natural that in the control group the "vascular resource" was exhausted more quickly.

Analysis of primary assisted patency (Fig., f) showed that the probability of AVF thrombosis is much lower in the late stages after the first intervention for CVS in the experimental group: repeated operations were performed in connection with increasing manifestations of venous hypertension (clinical manifestations, indirect "dialysis" signs: decreased HD effectiveness, increased pressure in the venous line, increased circulation in the vascular access). If the second operation was performed shortly after the first intervention for CVS, the differences between the groups are not so obvious: the P value is very close to the threshold of statistical significance ($p = 0.033$ according to the Breslow-Day test). When analyzing the functional primary assisted patency (i.e., when the starting point for the period corresponds with the start of AVF use), the results are somewhat different (Fig., e): the time interval between the start of AVF use and the first intervention for CVS compensates to some extent the differences between the groups. Differences in the long-term period are statistically insignificant (log-rank test $p = 0.0854$), but significant in the short term (Breslow-Day test $p = 0.0211$). However, both estimates are on the threshold of statistical significance. In other words, BA allows to slightly reduce the risk of AVF thrombosis. However, their effectiveness decreases as the duration of AVF use increases. At the same time, BA more than halved the risk of thrombosis in the early stages of its use – in the control group, the first AVF thrombosis occurred after 10 months, in the experimental group – after 21 months.

STUDY LIMITATIONS

First, the study was retrospective. Secondly, inclusion and exclusion criteria were determined to best achieve the research objective but limit the specific sample. Care should be taken when attempting to interpolate the resulting AVF patency estimates to the total HD patient population. The work was carried out to investigate the peculiarities of cause-effect relationships (which, in general, are relevant for the general HD patient population), and not to conduct a general assessment of the effectiveness of balloon angioplasty. Thirdly, the study did not include patients who used various stenting options. There is convincing evidence in favor of the fact that the use of stents can significantly increase patency [25, 63–68]. The main deterrent to the use of stents is the limited increase in primary patency, lack of clear indications for the use of stent and the choice of stent, as well as high cost of treatment [69]. Analysis of the effectiveness of angioplasty using stents requires a separate thorough investigation, which will be presented by us later. Fourth, we did not take into account the type of balloon, its working pressure and the extent of stenosis. There is reason to believe that these factors also have clinical significance [66, 70–72]. Fifth, we did not include in the study patients in whom AVF was created using a synthetic vascular prosthesis, as well as those patients in whom prosthesis was used during reconstructions (such patients were excluded from the study). This is an important factor in the context of our study, since it is obvious that a vascular prosthesis has less potential for significant increase in arteriovenous blood flow compared to native AVF.

CONCLUSION

Unfortunately, it must be recognized that CVS inevitably leads to loss of vascular access from the ipsilateral side. Balloon angioplasty, at the moment, is virtually a non-alternative way to quickly restore central vein patency in patients on HD. They allow to slightly extend the period of AVF use. However, BA outcomes significantly depend on the time interval between the start of AVF use and CVS appearance. At the same time, percutaneous balloon angioplasty is not able to radically change the long-term outcomes of CVS. If this complication develops, it is necessary to assess the possibility of forming a new vascular access from the contralateral side. Multiple repeated balloon angioplasties are apparently justified in patients in whom the possibility of creating a new vascular access is doubtful.

AVF volumetric flow rate is an important factor determining the severity of clinical manifestations of CVS and the need for repeated surgical interventions. AVF blood flow reduction is an effective palliative treatment for CVS.

The authors declare no conflict of interest.

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DEPENDENCE OF MECHANICAL PROPERTIES OF MITRAL VALVE ANNULOPLASTY RINGS ON ANNEALING MODES

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Objective: to investigate dependence of the mechanical properties of mitral annuloplasty rings on heat annealing modes. **Materials and methods.** The study evaluates the nature of change in stress–strain curves under uniaxial compression of experimental samples processed at varying annealing temperature, duration and pressure. **Results.** It was noted that higher exposure, temperature, and lower pressure led to increased structural rigidity and strength for small strains. Moreover, the extent of influence of annealing temperature and duration was comparable. A 40% (500–700 °C) change in temperature altered the mechanical properties of the ring – 20% increase in strength. A similar change in heat treatment time (4.5–6.5 min) resulted in a 27% increase in the force required for a 15% compression. **Conclusion.** The experimental dependences presented in the work allow recommending main parameters for heat treatment mode: temperature range 600–700 °C, 10.5 minutes exposure time, and 0.1–0.5 atm air pressure in the furnace chamber.

Keywords: nitinol, annealing, mitral regurgitation, annuloplasty, prosthetic ring.

INTRODUCTION

Chronic ischemic mitral regurgitation (IMR) is quite common and significant complication of myocardial infarction accompanying it in 20–30% of cases [1, 2]. The pathophysiological mechanism of IMR includes adverse remodeling of the left ventricle, dilatation of the fibrous ring and restriction of leaflet mobility, due to, among other reasons, changes in the geometry and properties of the chordal-papillary apparatus [3]. Such conditions require correction, both of ischemia as the root cause, by the technique of myocardial revascularization, and directly of morpho-functional disorders of the mitral valve by prosthetics or annuloplasty [4]. Modern publications and meta-analyzes of large studies comparing prosthetics and mitral valve reconstruction cannot definitely recommend this or that approach [5–7]. In general, most of these studies conclude that there are no significant differences in survival rate, the frequency of deaths associated with the intervention, or the frequency of serious adverse cardiac or cerebrovascular events, focusing on the benefits only for individual groups or for individual indicators [6, 7]. Thus, the choice of optimal surgical tactics for the correction of severe IMR in routine practice depends on a number of clinical and subjective indicators.

Modern trends in the development of rings for mitral annuloplasty are aimed at providing a compromise biomechanics of the fibrous ring with minimizing the stress-strain state in the relaxation phase to minimize the risk of mismatch between the characteristics of the product and surrounding tissues. Such an approach can maximally preserve the three-dimensional architectonics and mobility of the mitral fibrous ring with the possibility of natural deformations during the cardiac cycle and positively effect reduced risk of complications in the form of detachment of the implanted ring and subsequent fistula formation [8–10]. Three types of rings are distinguished by their stiffness: semi-rigid, rigid, and band. Current studies show that in the early postoperative period, semi-rigid rings have advantages over rigid rings; however, long-term clinical observation results show these advantages become less noticeable [6]. Nevertheless, further improvement of approaches and the introduction of new materials in the construction of semi-rigid rings aimed at maintaining the mobility of the fibrous ring can increase their efficiency in the correction of IMR and affect the clinical results of application [11–13]. In this connection we, in the FGBNU (Federal State Budgetary Scientific Institution) Research Institute of Complex Problems of Cardiovascular Diseases, are developing our own design of the mitral valve ring for IMR cases based on a semi-rigid support frame of a material with super elastic properties (nitinol).

MATERIALS AND METHODS

Target of research

The study was targeted at the supporting frames of the designed ring, closed three-dimensional ellipsoidal

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wire structure of medical titanium nickelide (SE508L-VM). The initial concept of the support ring involves semi-rigid execution in a compartment with a closed loop, which, on the one hand, will ensure its mobility in the systole-diastole cycle, and on the other hand, will reliably maintain the shape of the fibrous ring. All experimental ring frames were made in 30 mm size defined as a longitudinal length. The final form of the supporting frames was given by heat treatment in a metal matrix-mandrel, fixing the three-dimensional geometry of the product. The heat treatment as such was performed in TVF1200X43 (Aktan Vacuum LLC, Russia) tube muffle furnace able to create reduced pressure in the modes shown in Table.

In general, the mode selection for final shaping the support ring included a variation in the temperature (500–700 degrees) and time (4.5–12.5 min), and pressure (0.1–1.0 atm) indicators of heat annealing. In addition, in order to establish the basic dependences of the strength and elastic strain properties on the wire diameter, the supporting frames were made of 0.48 and 1.00 mm wire.

As a control, commercial size 30 semi-rigid rings were used: Physio (Edwards LifeScience, USA), CG Future™ (Medtronic, USA), Memo 3D™ (LivaNova, UK), widely used in surgical practice [14–16].

Table

Characteristics of the annealing modes of the studied annuloplasty rings

Nos.	t, °C	T, min	P, atm	D, mm
1	700	6.5	0.1	0.48
2	600	6.5	0.1	0.48
3	500	6.5	0.1	0.48
4	700	12.5	0.1	0.48
5	700	10.5	0.1	0.48
6	700	8.5	0.1	0.48
7	700	6.5	0.1	0.48
8	700	4.5	0.1	0.48
9	700	6.5	1.0	0.48
10	700	6.5	0.5	0.48
11	700	6.5	0.1	0.48
12	700	6.5	0.1	1.00
13	700	6.5	0.1	0.48

Test procedure

The selection criteria criterion for the optimal mechanical parameters was the physical and mechanical properties of the ring frameworks under uniaxial (longitudinal and transverse) compression. The mechanical properties were measured on a Z-series universal testing machine (Zwick/Roell, Germany) with a 50N nominal force sensor. The test samples were mounted between flat plate holders with subsequent application of a load until 15% strain was achieved (Fig. 1). The load range was chosen empirically as the elastic strain area. Loading and unloading were performed at 50 mm/min. meanwhile, the data on the "force-offset" ratio were obtained, serving to analyze the key mechanical property, i.e. rigidity of the frames.

RESULTS

Assessment of physical and mechanical properties

The main dependences of the mechanical properties of the samples under study on pressure, temperature, and heat treatment time are presented in Fig. 2.

The results of the study show that an increase in exposure, temperature, as well as decrease in pressure led to an increase in structural rigidity and strength in the range of small strains. Similar trends were shown for the transverse direction, however, to a lesser extent as the applied strain was lower, 3.2 mm versus 4.9 mm for longitudinal direction.

The dependence of the mechanical properties of the samples on the wire diameter of 0.48 compared to 1.00 for the same conditions showed the expected increased stiffness for the second option; however, the increase in

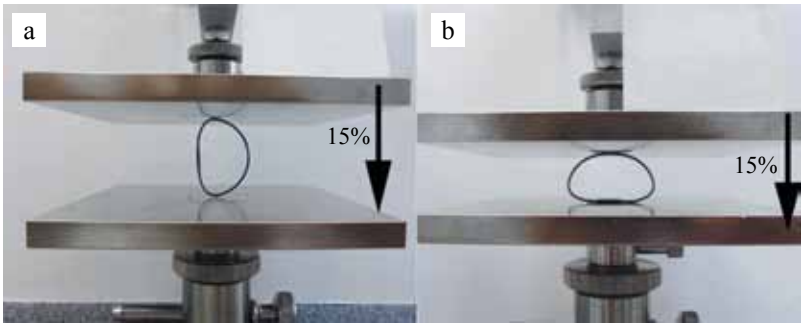

Fig. 1. Mechanical testing of the annuloplasty rings: a – mitral annuloplasty ring mounted between grips of the universal testing machine; b – a similar ring tested in the transverse compression (baseline)

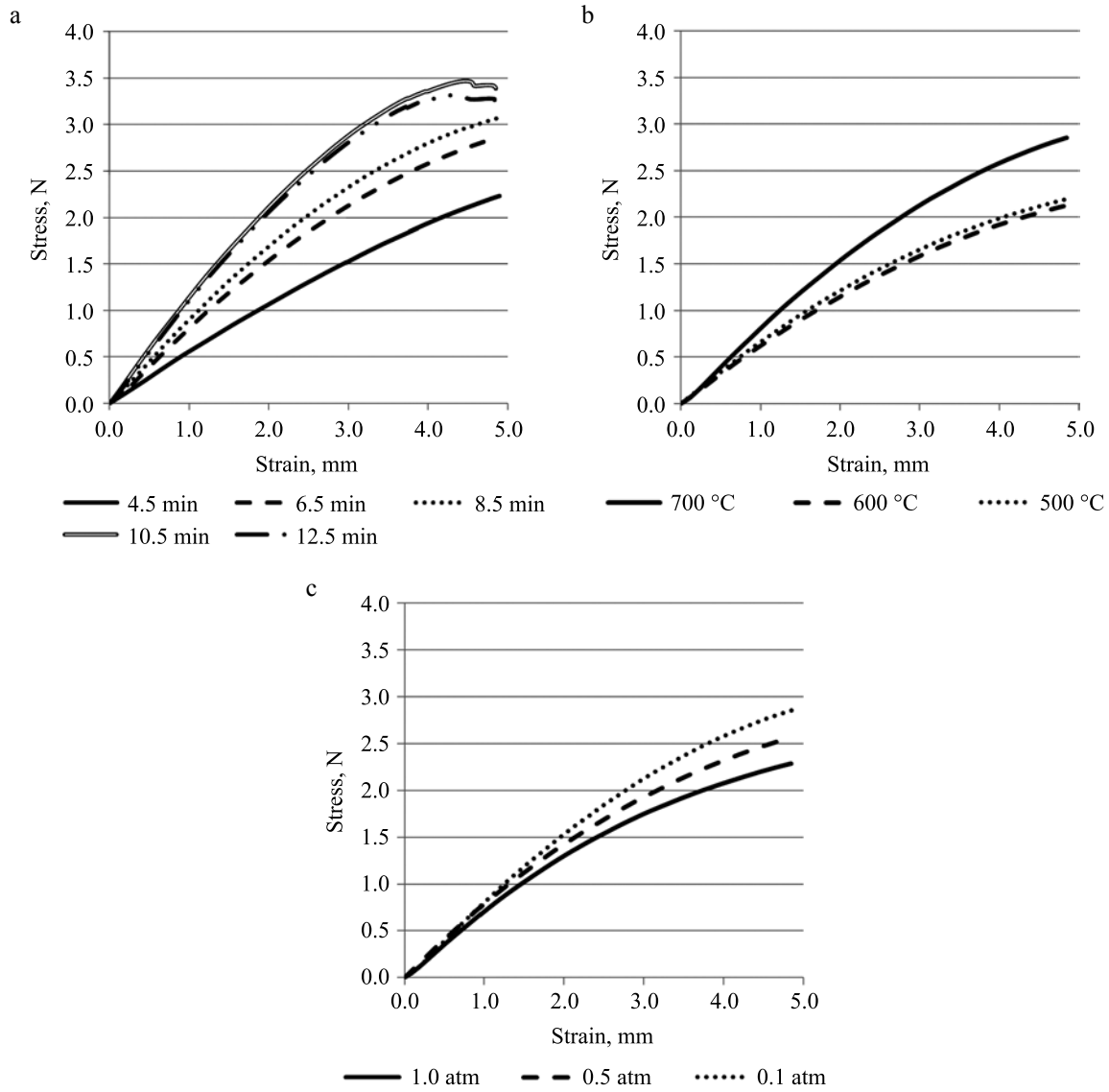

Fig. 2. Dependencies between the mechanical properties of the tested samples and the annealing modes, presented as the stress-strain curves under uniaxial compression in the longitudinal direction by 15%: a – processing time; b – annealing temperature; c – pressure, atm, in the furnace chamber

compression force was disproportionate to the increase in wire thickness. So, with an increase in diameter by 2.08 times, the force required for compression increased by 9.7 times, from 2.44 N to 25.9 N. A similar increase in strength was obtained for the transverse test – by 8.6 times. In this case, the plastic deformation of 38.5% was noted.

DISCUSSION

In general, the results of the study demonstrated a persistent dependence of the properties of the product on the heat annealing mode. Moreover, the degree of influence of temperature and time was comparable in terms of contribution. In the first case, a change in the parameter by 40% (500–700 degrees) caused a change in the mechanical properties of the ring in the form of strength increase by 20%. A similar change in annealing time (4.5–6.5 min) caused a 27% increase in the force required for compression by 15%.

However, it is clear that the variability within each parameter is heterogeneous. An increase in the thermal exposure time by 2 min in the range from 4.5 to 10.5 min always led to an increase in the rigidity of the support ring (Fig. 2), while the transition of 10.5 to 12.5 minutes made no significant changes in the mechanical properties for either the longitudinal or transverse directions. Apparently, a change in the properties of the material with an increase in the heat annealing time is determined only by the metal volume that has time to warm up to the required temperature. Given the high heat capacity, with a short duration of heat treatment, the mandrel matrix did not have time to sufficiently heat up, which led to insufficient heating of the ring itself. The heat treatment process consists in giving the product a

new "parent" form without changing the configuration of the crystal lattice [17]. It can be assumed that with a small exposure, an insignificant part of the material of the ring framework under study did not have time to fix the necessary "parent" shape, which, on the whole, did not visually express in the ring geometry but affected its physical and mechanical properties. Thus, for thy present study, it follows that a minimum of 10.5 minutes is sufficient to completely warm up the mandrel matrix and transfer heat energy to the ring itself to completely fix the "parent" geometry when using titanium nickelide wire with a diameter of 0.48 mm.

The features of the physicomechanical response of nitinol are determined by the transformation of the phases of the austenite-martensite crystal lattice and vice versa, providing a high percentage (up to 9–10%) of reversible strain [18]. The determining factor of this transformation is the transition point A_f (Austenite Finish – the final transition to austenite)б the temperature at which the material demonstrates its basic physical and mechanical properties when it is in the austenite phase, i.e. in working order. An important feature is an increase in the stiffness of the material depending on the difference between A_f and the current material testing temperature [19]. For example, at $A_f = 0$ °C and testing at room temperature of 22 °C, the alloy will exhibit greater rigidity than an alloy with $A_f = 17$ °C. It has been shown in the literature for nitinol that the A_f offset is affected primarily by the treatment temperature. Fig. 3 shows that the temperature of the heat annealing significantly shifts A_f, especially after 600 °C [20].

A similar result was obtained in the present study when the temperature changed from 500 to 600 degrees: the properties of the frames did not significantly change, and with an increase to 700 degrees a sharp increase in compression force and rigidity was observed. This effect is due to the characteristics of A_f which can be significantly shifted precisely by high temperature in the range of 600–700 degrees. The heat treatment process itself is also possible at lower temperatures, ranging from 450–550 degrees [17, 22]; however, for this range, on the contrary, an increase in A_f is shown to lead to decrease in stiffness.

Another factor influencing the parameter A_f is the molar composition of the alloy, namely, the nickel – titanium balance [17]. One per cent change in the concentration of free nickel or titanium leads to a shift in the temperature of transition to austenite by 100 °C [23]. At the same time, the air pressure in the furnace chamber which determines the oxygen content during heat treatment and, ultimately, the amount of nickel and titanium oxides formed, indirectly affects the change in the composition of the nickel-titanium alloy. The present study showed that during heat treatment under conditions of normal atmospheric air pressure, a weaker structure was obtained in comparison with the options under reduced pressure (0.1 and 0.5 MPa). The reason for this phenomenon is a decrease in the percentage composition of free nickel and titanium due to the formation of oxides NiO, Ni_2O_3, and TiO_2, i.e. in changing the equibalance state of nickel-titanium. In the initial alloy, the amount of nickel is 50.8% by mass [24] featuring its physicomechanical response, the effect of superelasticity [25]. In the process of oxidation, a change occurs, usually uncontrolled, of the nickel-titanium ratio, which changes the mechanical characteristics due to the offset of the A_f point. Thus, the

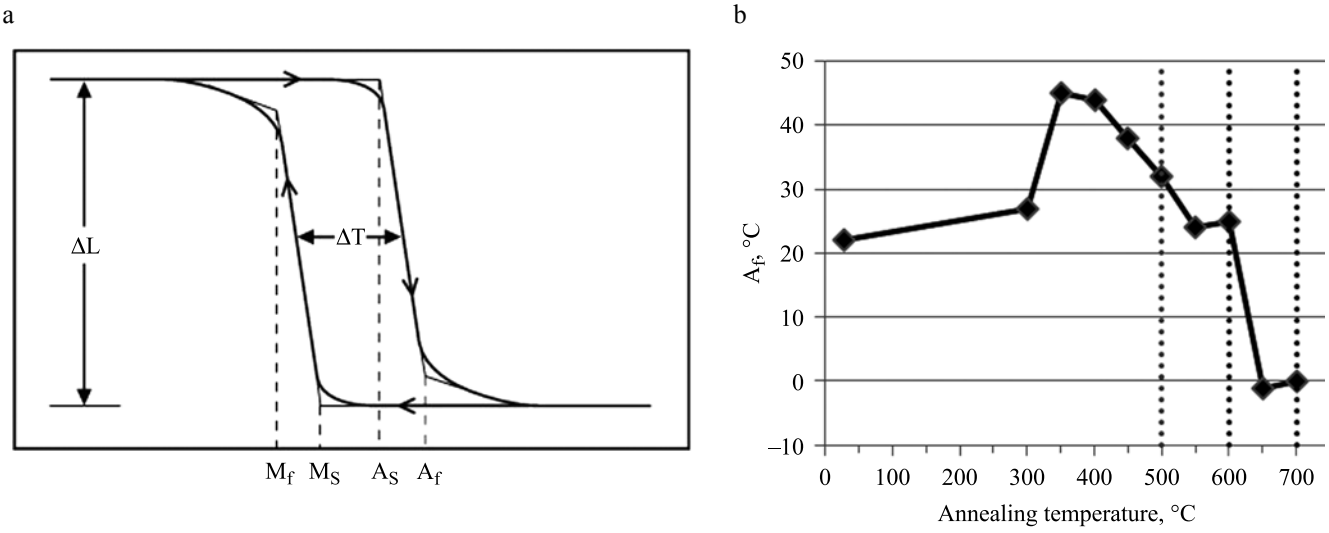

Fig. 3. Nitinol response to temperature: a – the dependence of the nitinol phase state on the temperature; A_s, the initial temperature of martensite to austenite transformation during heating; A_f, the final temperature of martensite-austenite transformation during heating; M_s, the initial temperature of austenite-martensite transformation upon cooling; M_f, the final temperature of austenite-martensite transformation upon cooling [20]; b – A_f offset under the annealing temperature of Ti-50.85 mol % Ni [21]. The dotted lines indicate the annealing modes used in this study: 500, 600 and 700 °C

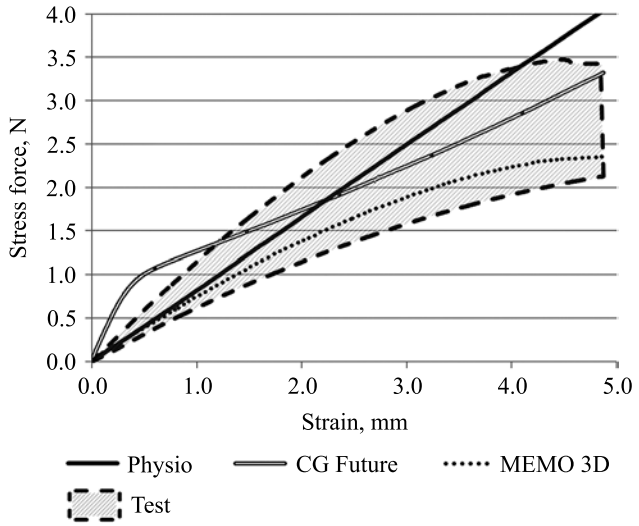

Fig. 4. Analysis of the variability of the mechanical properties of the study samples in comparison with the 30-mm commercial annuloplasty rings: Physio (Edwards LifeScience, USA), CG Future™ (Medtronic, USA), Memo 3D™ (LivaNova, UK)

occurrence of oxides can significantly distort the predicted properties of the final product, increase or decrease its rigidity, which is a negative factor in real production and most likely is leading to rejection of frames with properties altered due to oxidation. It is worth noting that the occurrence of oxides is confirmed visually, by changing color of the surface of the frames to gray, i.e. the formation of titanium oxide TiO_2 [26], which was seen on the frames under study at heat treatment under normal pressure.

Comparison of the physicomechanical properties of the support frames under study with foreign commercial prosthetic rings of similar diameters and purposes showed the similarity of physicomechanical properties at 15% compression (Fig. 4). The clinical results of commercial semi-rigid rings (Physio, CG Future, Memo 3D) [14–16], which are already well established in surgical practice, are presumably due to their elastic deformation properties similar to those of native tissues. With this in mind, it is possible to assume that the developed prosthetic rings will have similar biomechanics.

CONCLUSION

In general, the present study made it possible to determine promising heat treatment modes for further production, taking in mind the conformity with similar commercial devices, annuloplasty rings of world manufacturers. The obtained experimental dependences demonstrate the advantages of the following parameters of the heat treatment mode: temperature range of 600–700 degrees, exposure time from 10.5 minutes, air pressure in the furnace chamber of 0.1–0.5 atm.

The study was performed as part of the fundamental theme of the Research Institute for Complex Problems of Cardiovascular Diseases, Kemerovo, Russian Federation No. 0546-2015-0011 Pathogenetic substantiation of the development of implants for cardiovascular surgery based on biocompatible materials, with the implementation of a patient-oriented approach using mathematical modeling, tissue engineering and genomic predictors.

The authors declare no conflict of interest.

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RESULTS OF CORRECTION OF AORTIC VALVE DEFECTS USING SMALL-DIAMETER "BioLAB" XENOPERICARDIAL PROSTHESIS IN OLD PATIENTS

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A prosthesis-patient mismatch (PPM) describes a state in which the valve prosthesis implanted during surgery is too small in relation to the patient's body size. This leads to high transvalvular pressure gradients. We investigate direct results and dependence of transvalvular pressure gradients on body mass index and surface area in patients after correction of aortic valve defects using small-diameter BioLAB prosthesis. **Material and methods.** From January 2011 to August 2018, 65 small-diameter (18, 20) BioLAB scaffold xenopericardial prostheses were implanted in aortic position at the Department of Emergency Surgery for Acquired Heart Defects, Bakulev National Medical Research Center of Cardiovascular Surgery. The average age of the patients was 75.4 ± 4.1 (65–86 years). The average patient body mass index was 25.74 ± 5.11 kg/m^2 (19.57–39.54). The average body surface area was 1.79 ± 0.15 (1.54–2.18). **Results.** Isolated aortic valve replacement was performed in 38 (58%) patients, the rest of the surgeries were combined with other techniques. There were no reoperations due to early prosthetic endocarditis or prosthetic dysfunction in hospital. Hospital mortality was 6% (4 patients). Correlation dependence of peak pressure prosthesis gradient on body surface area and body mass index was 10% and 8%, respectively. **Conclusions.** This study confirmed the safety and effectiveness of using small-diameter BioLAB scaffold xenopericardial prostheses in aortic valve position.

Keywords: aortic valve replacement; bioprosthetic replacement; prosthesis-patient mismatch.

Over the past decades, as a result of aging population in general, an increasing number of elderly patients are referred to aortic valve replacement surgery. Biological prostheses are strongly recommended for this cohort, since they provide freedom from lifelong administration of indirect anticoagulants and potential durability, which provides better survival without valve-dependent complications compared to mechanical prostheses [4].

In 2004, the Bakulev National Medical Research Center of Cardiovascular Surgery (Moscow, Russian Federation) was the first in Russia to create a low-profile xenopericardial prosthesis on a rigid frame for an aortic position of 18, 20, 22 and 24 mm. The first experience with prosthesis implantation showed good immediate results and excellent hemodynamic parameters in the early postoperative period [1]. Early results showed significant regression of left ventricular myocardial mass in patients with critical aortic valve stenosis and severe left ventricular hypertrophy [2].

Our report analyzes the experience of using the small size BioLAB scaffold xenopericardial prosthesis in elderly patients, considering the peculiarities and risks of the hospital period, the occurrence of structural valvular degeneration, non-structural dysfunctions and endocarditis after surgery.

MATERIALS AND METHODS

January 2011 to August 2018, the emergency surgery department of acquired heart defects implanted 65 BioLAB frame xenopericardial prostheses of small [18, 20] size into the position of the aortic valve; one patient received a size 20 prosthesis twice, the second time due to late prosthetic endocarditis. The average age of patients was 75.4 ± 4.1 (65–86). Of 64 patients, there were 53 (85%) women, 10 (15%) men. In addition to general clinical examination methods, all patients underwent coronarography before surgery. According to the study results, the problem of the need for coronary artery bypass grafting was resolved. Operational mortality was defined as the death of patients during hospitalization. Postoperative serious complications worsening the prognosis of the course of the underlying disease were considered mediastinitis, prolonged ventilation, pneumonia, and rethoracotomy. BSA and BMI were measured in all the patients. Weight was considered insufficient with the BMI less than 20 kg/m^2, normal BMI from 20.0 to 24.9 kg/m^2, and excessive with BMI from 25.0 to 29.9 kg/m^2, obesity was stated with BMI of 30.0 to 34.9 kg/m^2 and morbid obesity with BMI of over 35 kg/m^2.

Transthoracic echocardiography was performed on all patients before surgery and in the early postoperative pe-

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riod (10–30 days after surgery). To calculate the average and peak systolic gradients on the aortic valve, a modified Bernoulli formula was used. In the postoperative period, in addition to gradients on the aortic prosthesis, the effective area of the hole, the indexed area of the hole, the mass of the left ventricular myocardium and the indexed mass of the left ventricular were measured. All measurements of peak and systolic gradients on the aortic prosthesis were performed at blood pressure indices of 110–130/70–90 mmHg and HR of 60–90 bpm. The studies were performed on SiemensAcuson and HewlettPackard Sonos-2500 devices with sector phase-electronic sensors with 2.5 and 3.6 MHz frequencies.

Statistical analysis

The statistical analysis was carried out with standard procedures (Student t-test, Mann–Whitney U-test, Chi-square test); for quantitative indicators, the mean value (M), standard deviation (SD), maximum and minimum values, median, and IQR were defined.

RESULTS

The characteristics of all operated patients are presented in Table 1. The average BMI was 25.74 ± 5.11 kg/m^2 (19.57–39.54). According to our classification used by us, 19 patients were overweight, 12 obese (BMI over 30 kg/m^2) and 8 had morbid obesity with BMI more than 35 kg/m^2. All operations were performed under cardiopulmonary bypass and pharmaco-hypothermic cardioplegia. The main access used was median sternotomy, six patients (9%) underwent a ministernotomy (j-shape). The indications for access were obesity in three patients (BMI 35.43; 39.54 and 33.79), obstructive pulmonary disease with decrease to 47% and osteoporosis. The patients were aged 70, 78, 79 (two patients), and 86 (two patients). All patients underwent an isolated aortic valve replacement. In three patients, the sternotomy was repeated due to previously performed interventions (aortic valve mechanical prosthesis, aortic valve BioLAB scaffold xenopericardial prosthesis, and bypass (anterior interventricular). One patient was previously implanted with the prosthesis in the aortic position endovascularly, regrafted as the result of prosthesis degeneration after four years (Table 2). 4 prostheses of size 18 and 61 of size 20 were implanted.

There were no reoperations associated with early prosthetic endocarditis or dysfunction at the hospital stage. One patient was reoperated 15 months after surgery due to late prosthetic endocarditis; she was implanted with a size 20 "BioLAB" frame xenopericardial prosthesis. The operation went as planned, without postoperative AEs, and the patient was discharged from the department on the 10th day in a satisfactory condition.

The average resuscitation time spent in the ICU was 48.1 ± 38.7 h (19 to 521 h).

Table 1

Demographic indicators and risk factors (n = 65)

Parameter	Abs. (%)
Mean age	75.4 ± 4.1 (65–86)
Over 75	39 (60%)
Gender, f/m	55(85%) / 10 (15%)
BMI (kg/m^2)	25.74 ± 5.11 (19.57–39.54)
BSA (m^2)	1.79 ± 0.15 (1.54–2.18)
Arterial hypertension	28 (44)
Diabetes melitis	13 (30)
CVA in history	3 (5)
COPD	11 (17)
LVEF (%)	61.96 ± 5.9 (40–80)
FC III–IV NYHA	65 (100)

Table 2

Surgical interventions and intraoperative parameters (n = 65)

Intervention	Abs. (%)
Aortic valve replacement (AVR)	38 (58%)
AVR + mitral valve plasty	9
AVR + tricuspid valve plasty	6
AVR (ministernotomy)	6 (9%)
AVR + myectomy	3
AVR + CABG	14 (22%)
Regrafting (+ after TAVI)	3
*CPB (min)	120 (41)
*Aortic compression time (min)	76 (12)

Note. * – median (IQR).

Table 3

Hemodynamic parameters of the early postoperative period (n = 61)

Parameter	Mean (range)
ESV	34.5 ± 12.0
EDV	99.8 ± 28.4
LVEF	61.54 ± 8.53
Peak, mmHg	16.6 ± 5.7 (6–27)
Mean, mmHg	8/6 ± 3.4 (2.8–17)

The analysis of hemodynamic parameters showed that the maximum gradient on the size 20 prosthesis was 27 mm Hg (peak) and 17 mm Hg (mean systolic) in one patient with the BSA of 1.83 and BMI of 24.51 kg/m^2. We studied the effect of BSA and BMI on the peak gradient of the aortic prosthesis in the early postoperative period (Table 3). The correlation dependence turned out to be very low and reached 10% and 8%, respectively (Fig. 1, 2).

At the hospital stage, there were 4 (6%) lethal casts with the ages of 71, 75, 77 and 86. The causes were not related to the prosthesis type. One of the patients who died, aged 77, had the BMI of 19.57 and was the only one with cachexia. She endured the operating period

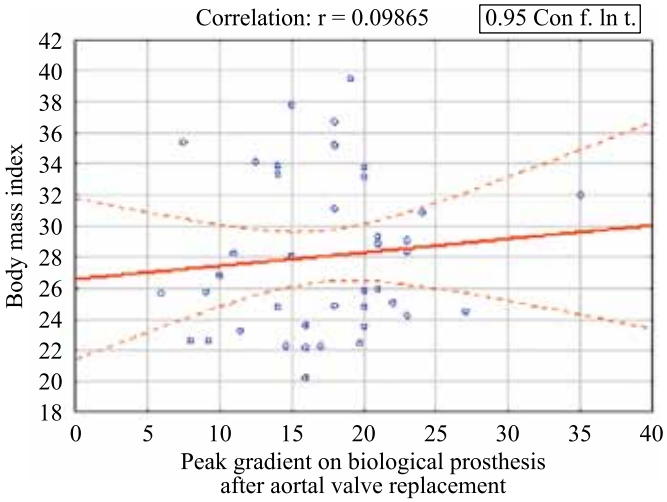

Fig. 1. Dependence of the peak gradient of the aortic prosthesis on the patient's BMI (correlation dependence 10%)

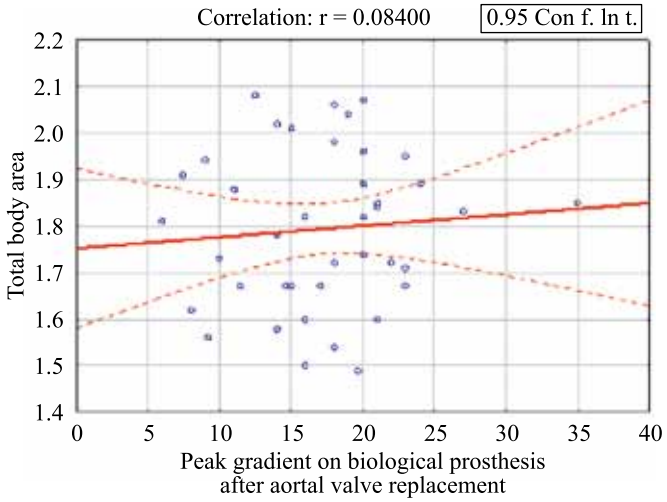

Fig. 2. Dependence of the peak gradient of the aortic prosthesis on the patient's BSA (correlation dependence 8%)

well, was transferred to the ward on the 2nd day, however, physical activity was restoring extremely slowly, and on the 9th day of being in the ward, her condition worsened, pneumonia was diagnosed, and the patient was transferred to the ICU with respiratory failure. Further, heart and multiple organ failure progressed. One patient, operated at the age of 86, with the BMI of 21.26, also went through a smooth postoperative period, was transferred to the ward, but at night of the 7th day there was a sudden cardiac arrest followed by resuscitation which was complicated by multiple organ failure. Another 75-year-old patient at the stitching stage of the sternum experienced massive bleeding (1.5 L) from the aortotomy incision, the sternum was reconstituted, artificial circulation was connected, and the aortotomy defect was sutured. However, due to prolonged aorta clamping and time of cardiopulmonary bypass, multiple organ failure progressed, and the patient died. Another patient, 71 years old, died of multiple organ failure; she was operated for post-radiation heart damage (aortic stenosis and coronary heart disease), a long and traumatic operation was the result of the initial severity, a large amount of surgery, a long period of aorta clamping and cardiopulmonary bypass.

DISCUSSION

In Europe, the prevalence of aortic stenosis is 3–8% among people over 75. It is known that in the absence of treatment in 90% of patients with severe aortic stenosis, the expected life expectancy does exceed 10 years, and 50% of patients die within 2 to 3 years after the onset of the disease symptoms [11]. Calcined aortic stenosis, i.e. the formation of fibro-calcium nodules on valve structures, has a prevalence of 0.4% in the general population and 1.7% in the population of people over 65 [12]. The degenerative disease has become the most common cause of aortic valve disease in developed countries. These changes are no longer considered a benign consequence of aging, valve calcification is the result of an active process, which, like atherosclerotic vascular disease, caused by destruction of the base membrane

between the endothelium and connective tissue of the leaflet, inflammatory cell infiltration, and lipid deposition. Diabetes, hypercholesterolemia, hypertension, and smoking [13] are still the risk factors.

Aortic valve replacement in the treatment of aortic stenosis is one of the most common heart valve operations performed in current cardiac surgery. Typically, mechanical valves, which are more thrombogenic but also more durable, are implanted in patients under 65, while biological valves are mainly used in older people. The problem of tissue valves is well known, it is their tendency to degenerate [14]. However, more than half of the valves used in the world to replace the aortic valve are bioprostheses, as a result of the patients' preference and an increase in the number of long-living persons in the general population, especially after the the TAVI technique was introduced in 2007. Predictors of bioprosthesis tissue degeneration include patient-related factors (younger age, higher body mass index), cardiovascular and related factors (smoking, diabetes, dyslipidemia, renal failure, left ventricular hypertrophy, and small prosthesis size). An analysis of the long-term results of implantation of 12,569 Carpentier-Edwards scaffold xenopericardial prostheses showed that for patients under 60, the reoperation risk associated with degeneration in 10, 15 and 20 years was 5.6% (95% confidence interval [CI], 4.7–6.8), 20% (95% CI, 17–23) and 45% (95% CI, 39–52), for patients of 60 to 80 – 1.5% (95% CI, 1.3 to 1.7), 5.1% (95% CI, 4.4 to 5.8) and 8.1% (95% CI, 6.7 to 9.7), respectively, and for patients over 80 – 0% (for the entire observation period). Earlier, we reported that the indication for implanting a biological prosthesis in the aortic position is the age over 65 [3]. However, now we consider the indication for implantation of a bioprosthesis into the aortic position to be over 70, therefore in this study the average age of patients is 75.4 ± 4.1 years, and 60% of them are over 75.

Many researchers believe that the small size of the prosthesis is not directly tied to the risk of degeneration and explantation of the bioprosthesis in the long term, but a problem that must be considered when implanting the small size of the prosthesis in the aortic position is the probability of a mismatch between the prosthesis and the patient (PPM), which was first described in 1978 by Rahimtoola [9]. Douglas R. Johnston et al. investigated the effect of the peak gradient on the prosthesis in the early postoperative period on long-term results and, in particular, the relationship of indicators with the prosthesis degeneration. It turned out that high peak gradient values affected bioprosthesis degeneration to a greater extent in young patients and were not associated with biological tissue degeneration in patients over 80. An increase in the peak gradient by more than 10 mmHg from the normal value in the early postoperative period in young patients was associated with a more than twofold-increased risk of explantation associated with degeneration of biological tissue 20 years after surgery [14]. In the present study, despite the use of only small sizes of frame xenopericardial prostheses, including patients with obesity and a large BSA, the average peak gradient was 16.6 ± 5.7 (6–27) mm Hg and the average systolic gradient of 8.6 ± 3.4 (2.8–17) mm Hg, We investigated the dependence of the peak gradient on the patient's myocardial mass index and on the surface area in the early postoperative period. It turned out that the correlation dependence is extremely low: 10% and 8%, respectively. Zheleznev S.I. et al. [4] analyzed the results of implantation of 52 BioLAB prostheses of 20 sizes for old and greater age patients. In this group, average BMI was 28.1 ± 5.5 kg/m^2, i.e. slightly more than in the present study (25.74 ± 5.11 kg/m^2) and the average BSA was 1.6 ± 0.1. A study by the authors of trans-prosthetic gradients in the early postoperative period showed: peak gradient 23 ± 6 mm Hg, average systolic 12 ± 4 mm Hg, effective area 2.4 ± 0.2 sq.cm and indexed area of the hole 1.5 ± 0.2, which corresponds to good performance. Zheleznev S.I. et al. [4] conclude that the technical simplicity of implantation, the formation of a sufficiently large passage opening, and blood flows characterized by laminarity, predict the stability of the results in the early postoperative period.

The literature reports discuss the effect of the prosthesis size and the indexed area of the hole on the regression of left ventricular myocardial mass, which largely determines the quality of life in the long term and overall survival of patients. However, Rajappan et al. demonstrated that the degree of disturbance of the reserve of coronary blood flow measured by positron emission tomography was associated with the severity of valve stenosis before surgery, not with the mass of the left ventricle. Changes in coronary blood flow after aortic valve replacement were not directly related to regression of left ventricular myocardial mass, but were more dependent on the magnitude of the change in the indexed area of the valve opening as a result of aortic valve prosthetics [6, 7]. Garcia et al. [8] reported that if the index of the indexed area of the opening on the aortic valve exceeds 0.8–0.9 cm^2/m^2, then the reserve of coronary blood flow is practically unchanged but decreases sharply when the indexed area of the opening is below this threshold, and it becomes almost completely exhausted when the indexed area of the hole is less than 0.5 cm^2/m^2.

Basically, all the presented results of aortic valve replacement in elderly patients necessarily include a subgroup with coronary heart disease and coronary artery bypass grafting. The subgroup analysis shows differences in the results of aortic valve replacement with or without coronary artery bypass grafting, but these differences are not statistically significant [10]. In the present study, at the hospital stage, one patient died after correction of the combined pathology; however, it

was a patient with post-radiation damage to the heart and coronary arteries. It is known that this pathology is associated with pathological malignant calcification of the base of the heart and coronary arteries, which can lead to a more traumatic surgical process. In general, the treatment of concomitant pathology did not affect the length of hospital stay.

An important question remains open: the impact of BMI on the results of surgical treatment, both at the early stage and in the distant postoperative periods. In the present study, the average BMI was 25.7, which, according to the classification we used, should be classified as overweight. Many studies on patients with congestive heart failure have shown a remarkable association between patients losing weight before surgery and high mortality [16]. The mechanical weight loss associated with heart failure is explained as part of metabolic disorders, namely insulin resistance and excessive catabolic activity due to the release of catecholamines. As a result, the metabolic phenotype of heart failure is characterized by depletion of body tissues, including muscles, fat, and bones, leading to significant weight loss and, ultimately, to cachexia development. Therefore, a favorable prognosis for patients with obesity and heart failure suggests that they have higher metabolic reserves which allow them to better tolerate catabolic stress compared to patients without excess weight [17]. One of the 77 patients who died in our study had a BMI of 19.57 and was the only one with cachexia. She smoothly passed the operating period, but recovery of physical activity was extremely slow, respiratory failure due to muscular-respiratory dysfunction persisted, she felt worse on day 9, pneumonia was diagnosed, and the patient was transferred to the ICU. Further, heart and multiple organ failure progressed. In the present study, a J-shaped ministernotomy was performed for three obese patients in order to avoid the development of respiratory failure and wound complications in the postoperative period. It is known that ministernotomy with isolated aortic valve replacement is a safe procedure, it reduces the risk of blood loss, reduces postoperative hospital bed/day, patients suffer less from pain in the early postoperative period [18, 19]. Nobuyuki Furukawa et al. consider that the main advantages of the ministernotomy should be the reduction in postoperative pain, improved respiratory function, as well as an earlier return to daily activities which is significantly more important for elderly patients than the cosmetic effect of the procedure [20].

We believe that the present study has important clinical significance, as it supplements the accumulated experience and confirms the safety and efficacy of implementing small sizes of BioLAB frame xenopericardial biological prostheses in the aortic position. Based on this analysis, we can say that it is not necessary to resort to methods of expanding the fibrous ring in elderly patients with a narrow aortic root, as the desire to expand the fibrous ring to implant a larger prosthesis lengthens the perfusion time and may put patients at unreasonable risk.

The authors declare no conflict of interest.

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BIODEGRADABLE SMALL-DIAMETER VASCULAR GRAFT: TYPES OF MODIFICATION WITH BIOACTIVE MOLECULES AND RGD PEPTIDES

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The need for small-diameter grafts for replacing the damaged area of the blood pool is still very high. These grafts are very popular for coronary artery bypass grafting. Polymeric synthetic grafts are an alternative to autografts. A promising area of tissue engineering is the creation of a biodegradable graft. It can serve as the basis for *de novo* generation of vascular tissue directly in the patient's body. Optimization of the polymer composition of products has led to improved physicomechanical and biocompatible properties of the products. However, the improvements are still far from needed. One of the decisive factors in the reliability of a small-diameter vascular graft is the early formation of endothelial lining on its inner surface, which can provide atrombogenic effect and full lumen of the future newly formed vessel. To achieve this goal, grafts are modified by incorporating bioactive molecules or functionally active peptide sequences into the polymer composition or immobilizing on its inner surface. Peptide sequences include cell adhesion site – arginine-glycine-aspartic acid (RGD peptide). This sequence is present in most extracellular matrix proteins and has a tropism for integrin receptors of endothelial cells. Many studies have shown that imitation of the functional activity of the natural extracellular matrix can promote spontaneous endothelization of the inner surface of a vascular graft. Moreover, configuration of the RGD peptide determines the survival and differentiation of endothelial cells. The linker through which the peptide is crosslinked to the polymer surface determines the bioavailability of the RGD peptide for endothelial cells.

Keywords: tissue engineering, polymer graft, RGD peptides, endothelization, biocompatibility.

1. BACKGROUND

Cardiovascular diseases (CVDs) remain the main cause of mortality and disability for the population of most countries in the world [1]. According to WHO statistics, in 2015 CVDs caused the death of about 17.7 million people [2]. While analyzing this problem Mathers C.D. and Loncar D. estimated that by 2030 this number will be increased by 30% [3]. An unchallenged leadership among CVDs belongs to atherosclerosis, during the development of which atheromatous plaque develops and increases in the full thickness of the arterial walls [2]. This leads to an impairment in the vessel patency and consequently deterioration of the vascular supply to the tissues [2, 4–8].

In modern cardiovascular surgery during the treatment of the damaged vessel the choice lies between angioplasty and replacement by a vascular implant (vascular graft). Using the patient's own arteries and veins for implants presents an ideal option, however there are certain limitations in their use.

Auto-, xeno-, allotransplants

The patient's own arteries (thoracic and radial) and veins (great saphenous) can be used by many indications for transplants into the coronary bed [9]. The disadvantages of this type of transplants include: anatomic features of the vessel structure which prohibit its use for plastic material; limited number of arteries and veins; possible traumatization of both the vessel and the adjoining tissues during extraction; risk of ischemia development at the site of material sampling; age-related degeneration [10, 11]. At the same time allo- and xenografts are being developed. The leading problem related to allografts (homografts or transplants obtained from other people) and xenografts (transplants obtained from other animals) is foreign genetic material. The use of such grafts implies a carefully adjusted protocol of devitalization, aseptic processing and, if necessary, cryopreservation of the samples. Immune rejection, allergic reactions, infection process development and calcification – such complications may be expected in some cases when such implants are used [12–14].

Artificial transplants

Synthetic vascular grafts can be divided into 2 types: biostable and biodegradable. Biostable grafts are made from polytetrafluorethylene, polyethylene terephthalate, polyurethanes. Such prostheses are successfully used in reconstructive surgery for vessels over 6 mm in diameter. In case of an impaired vessel with a smaller diameter,

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the hemodynamics of which is characterized by a lower blood velocity, biostable grafts become inapplicable due to rapid hyperplasia of the neointima and thrombosis [15–17].

Biodegradable polymer vascular grafts are quite attractive. Their main special feature is imitation of the extracellular matrix structure with subsequent full replacement of the polymer matrix by the recipient's newly formed vascular tissue. The materials for manufacturing of such prostheses may include synthetic polymers, such as: polyglycolic acid, polylactic acid, polycaprolactone, polyglycerolsebacate, polyhydroxyalcanoates, etc. In order to produce tissue engineered vascular grafts various methods are used, such as solvent casting, phase separation, levigation from a polymer solution, 3D printing and electrospinning [18]. The latter may be considered a priority method. The electrospinning method may help achieve the stretching of the polymer solution into fibers the diameter of which varies between 10 microns to 50 nanometers, with the formation of various-sized and highly porous frames [19–21]. Also changing the manufacturing regimen and the solution formulation in the process of electrospinning enables to produce frames consisting of layers with different composition [22].

Improving biocompatibility of the synthetic material and modification types

Some polylactone type polymers demonstrate satisfactory mechanical properties, low toxicity and immunogenicity, however their highly hydrophobic nature and low surface energy limit the wetting properties of the material, adhesion and cell proliferation which are required for further tissue remodeling [23, 24]. Using a combination of synthetic and natural polymers (collagen, chitosan, fibrin, silk fibroin, polyhydroxybutyrate-covalerate etc.) can lead to the improvement of biocompatibility for the produced matrix [25–27]. Also the frame biocompatibility can be enhanced by means of using various polymers in the process of its manufacturing. Using a combination of polycaprolactone (PCL) with polyhydroxybutyrate-co-valerate (PHBV) demonstrated an increase in the biocompatibility of a matrix produced from these materials versus a sample made from only polycaprolactone [28].

At the stage of *in vivo* testing serious problems occur with biodegradable artificial prostheses: thrombogenesis, calcification, incompatibility of the physico-mechanical properties and compliance with the native vessel, inflammation process development, insufficient biocompatibility of the material [29]. Strategies aimed at overcoming such problems are focused, inter alia, on developing the biofunctional properties of the conduits.

In particular, stimulation of the graft inner surface endothelization may facilitate a decrease in the risk of thrombogenesis. The process of graft modification envisages including (incorporating into the nano size fibres of the polymer or surface immobilization) substances into the polymer matrix which promote adhesion retention, support the vital activity of the cells required for speedy formation of the endothelial lining and other *de novo* tissue formation. Such substances include a number of growth factors and chemoattractant molecules [30, 31]. At the same time, a significant scientific interest is related to surface modification of ready polymer matrixes by means of immobilizing functionally active peptides on their surfaces which are capable of selective adhesion to endothelial cells from the patient's system blood flow [32]. Such peptides include the arginine-glycine-aspartic acid (RGD) which is present in most of the extracellular matrix proteins [33]. The RGD sequence is one of the key ligands for integrins – receptors which are responsible for cell adhesion, migration, proliferation, differentiation and survival [34]. One of the key challenges in the development of devices with RGD-containing peptides is the choice of RGD configuration, as well as the ligand or linker by means of which the adhesive peptide will be immobilized on the polymer surface.

Currently the possibility of using RGD peptides for modification of the surfaces of constructions obtained by means of tissue engineering which come into contact with blood and require prompt surface endothelization is being studied simultaneously in many countries. Research groups carry out independent studies in this area, using their own protocols beginning with the synthesis of a certain peptide configuration and all the way through to a model of *in vivo* testing for a ready model. Therefore, according to existing literature data there is no evidence based opinion regarding a preferable configuration for an RGD peptide or the structure of a ligand/linker, which characterizes this area as under-investigated, and therefore quite attractive to be studied in regard to creating functionally active devices for the needs of cardiovascular surgery. The current review covers the main modern approaches used in the development of modified biodegradable prostheses with an emphasis on describing use of surface modification for small diameter vessel prostheses by means of RGD peptides.

Vascular endothelium

Vascular endothelium (VE) is a continuous highly differentiated monolayer of squamous cells of mesenchymal origin (endotheliocytes) which line the inner surface of each integral part of the cardiovascular and lymphatic systems [35]. Several distinctive features can be distinguished which emphasize the priority importance of achieving prompt and quality endothelization of the inner surface of a polymer vascular prosthesis. First of all, the endothelial monolayer is formed by endotheliocytes with various phenotypes the ratio of which depends on many factors: the amount of pressure in the vessel, the velocity,

the share stress force, a pulsing or constant flow, as well as peculiar features pertinent to the extracellular matrix [36, 37]. In other words, the endothelial lining of a vessel is a highly adaptive system which enables the vessels and cavities to support functioning under various conditions (types and length of the stimuli).

Secondly, the vascular endothelium has morphological and functional variations which match their specific location in the body [38]. The vascular endothelium produces a large amount of biologically active substances, from an inorganic molecule of NO to complex organic structures (C-type endothelial natriuretic peptides) [40, 41]. Thus, the VE is not only a barrier layer of cells between the blood (or lymph) and the subendothelial vascular tissues but also an active endocrine 'organ' which takes part in functional self-regulation, regeneration and remodeling of the vasculature, in direct metabolism of the tissues and organs, in transvascular substance and cell migration, for example leukocyte migration, as well as influencing the most important stage of the hemostasis system's work – coagulation [42–44]. The important contribution of the VE into the normal physiology of the body indicates that any disfunction in it may lead to a wide range of pathological conditions. The most socially significant and debatable ones among these are CVDs, sepsis and cancer [45–47]. Therefore, rapid formation of the endothelial monolayer on the inner surface of a biodegradable polymer prosthesis which replaces an impaired section of the vascular bed is a most important goal in the creation of biocompatible and functionally active vessel replacement devices. The speed and quality of the endothelization may determine the consistency of the tissue engineered prosthesis itself, its further remodeling, as well as the physiology of those tissues and organs (or organ systems) in the vascular pool of which it is implanted.

2. MAIN BIOLOGIGALLY ACTIVE SUBSTANCES USED FOR THE MODIFICATION OF TISSUE ENGINEERED VASCULAR PROSTHESES IN ORDER TO ACCELERATE THE INNER SURFACE ENDOTHELIZATION

Including substances capable to attract endothelial cells from the recipient's systemic blood flow, and to provide optimal conditions for their vital activity, into the tissue engineered matrix is one of the trends in creating biofunctional biodegradable vessel prostheses. Such substances include bioactive molecules which control polymer frame remodeling processes with a priority to rapid and quality endothelization of the inner surface. Much attention is devoted to growth factors – signal polypeptides which regulate cell survival, migration, proliferation and differentiation [48]. Due to the chemical instability of growth factors one of the common methods used to include them into the polymer matrix is incorporation. For example, in the process of two-phase electrospinning the biomolecules are enclosed into the polymer fibers which form the device, which ensures their structural integrity and prolonged release related to gradual degradation of the polymer fibre [49–51]. Another successful method which enables to ensure structural stability and increased lifespan for the molecules is the adsorption of growth factors to fibronectin, fibrin, gelatin, heparin, which in their turn are immobilized to the matrix surface. Speaking about tissue assimilation and remodeling at the polymer tube frame, the following fact should be taken into account: supporting vital activity for adhesive cells and future tissues is possible in case of availability of an extensive and branched vasculature [52]. Therefore the Vascular Endothelial Growth Factor (VEGF) as a modification component is quite interesting and indeed of prior importance, as it facilitates endothelization of the inner surface of the grafts as well as stimulating vascular network formation and growth on the transplant and capillary genesis throughout its thickness.

The VEGF molecule can ensure migration of already mature forms of the endothelial cells towards the polymer matrix from anastomosis zones and attract endothelial cell precursors from the blood [53]. The VEGF-A 165 isoform is most active in angiogenesis stimulation (prevailing numerically), being bound to the VEGFR2 receptor on the endothelial cell it provides most significant functional signals [54–56]. V.V. Sevostianova et al. (2018) published data describing the character of inner surface endothelization for polycaprolactone grafts with incorporated VEGF which have been implanted into the abdominal part of the aorta to laboratory rats for 1, 3, 6 months. Thus, PCL/VEGF grafts demonstrated better short-term (75% vs 50%) and long-term (100% vs 75%) permeability as compared to non-modified analogues. Due to VEGF use on the inner surface of the transplants already one month after implantation a large number of immature $CD31^+$ $CD34^+$ endothelial cells has been identified which during follow-up by the time of the implantation time into the abdominal part of the rats' aorta formed a monolayer with a prevalence of mature cells with $CD31^+CD34^-$ phenotype. Non-modified PCL grafts were not so successful [57]. Similar results have been obtained with co-polymer PHBV/PCL grafts modified by the same growth factor [58]. Research carried out by Henry, J.J.D. et al. (2017) showed that after implantation of vascular grafts from polylactic acid (PLLA – poly-l-lactide acid) into the carotid artery of laboratory rats and PLLA/PCL variation modified by VEGF in each case already two weeks later active angiogenesis has been noted: on the inner surface of 82% of the samples endothelial cells have been identified, while in non-modified graft this rate was 2 times lower [59]. Other bioactive factors make a less pronounced contribution to the endothelization process, acting more indirectly.

The basic fibroblast growth factor (bFGF) has an impact on many physiological and pathological processes: cell survival, differentiation, proliferation, angiogenesis, adhesion, as well as skeletogenesis and wound healing [60, 61]. BFGF angiogenesis is based on mature endothelial cell stimulation to proliferation and organization into tube structures [62, 63]. Both in *in vitro* and in *in vivo* experiments successful EC adhesion and viability results have been obtained. During cultivation of human microvascular endothelial cells (HMECs) and peripheral blood canine endothelial progenitor cells (CEPC) on the surface of a decellularized carotid artery of a pig covered with bFGF under conditions of blood flow imitation, more successful EC retention has been demonstrated on bFGF-modified samples (60%) [64]. Owing to the procedure of venous transplant wrapping in a bFGF-containing hydrogel sheet their structural and physiological properties have been improved, EC survival during implantation to laboratory mice has been improved vs. non-modified veins [65].

Many other growth factors and chemoattractant molecules are also used as agents for artificial polymer vascular graft modification. In particular the platelet-derived growth factor (PDGF) is interesting due to its participation during the embryonal and postnatal periods in differentiation, proliferation, migration of mesenchymal origin cells, in the formation and stabilization of blood vessels, in tissue regeneration [66–68]. The transforming growth factor beta (TGF-beta) when secreted into the extracellular environment by various cell types performs a number of functions, including cell proliferation and differentiation control as well as angiogenesis stimulation [69].

The stromal cell-derived factor – 1 alpha (SDF-1α) is a chemoattractant molecule which performs a number of important functions both in the embryonal period and in an adult body. SDF-1α controls migration of various cell types, attracts and takes part in the proliferation of endothelial progenitor cells from the bone marrow [70, 71]. Implantation of grafts made from polyester with SDF-1α into the carotid artery of sheep has shown stem cell attraction, improved endothelization, decreased intimal hyperplasia and thrombosis frequency [73].

During modification of low diameter synthetic vessel substitution devices several types of biologically active molecules can also be used to launch various effects stimulating and supporting endothelization, facilitating remodeling of vessel tissues with the formation of all the appropriate tissue layers found in a true vessel. Thus, layered incorporation of the VEGF, bFGF and SDF-1α complex into a biodegradable vessel graft made of PHBV/PCL promoted 100% permeability and early full graft endothelization in *in vivo* experiments vs samples with each factor incorporated separately [73]. It has been proved that bFGF and SDF-1α molecules supported sustainable VEGF-induced formation of quality endothelial lining on the inner surface of the vessel prostheses. High primary permeability during 12-month implantation to rats provided for the formation of vascular tissues in the place of the biodegradable matrix with simultaneous calcification intensity decrease and no immune rejection signs [74].

3. SURFACE RDG PEPTIDE MODIFICATION

A large number of methods aimed at the modification of the vessel substituting inner surface are being developed in order to get a functionally active endothelial monolayer. *In vitro* graft endothelization by autologous cells is an effective, however rather controversial method. In this case the time for producing a cell-colonized vessel graft and its cost are increased [75]. Such a graft can not be used in emergency cardiovascular surgery. In emergency cases a cell-less biodegradable graft which forms a microenvironment to attract cells that take part in the endothelization process can be a more successful option. Another area actively developed in tissue engineering is surface modification of polymer vascular prostheses which implies creation of a biomimetic surface the architectonics and functionality of which would be similar to that of the natural extracellular matrix.

The extracellular matrix and integrin receptors

The extracellular matrix (ECM) consists of a compound protein complex of various structure and configuration which is characterized by specificity of the ratio of the main glycoprotein – collagen – to other glycoproteins, proteoglycans and hyaluronic acid for each tissue type [76]. The main functions of the ECM are: forming borders between cell groups, creating a media for cell migration; regulating cell behaviour by means of growth factors and proteins containing cell adhesion sites. The combination of these functions also enables the ECM to support structural hierarchy in tissue organization [77]. Interaction between the ECM and the cells is carried out via integrin mediated cell adhesion. An integrin receptor is a heterodimer consisting of α- and β-subunits. Human cells have altogether 18 α-subunits and 8 β-subunits in different variations which represent 24 types of transmembrane receptors. Integrin reorganizes signals from the ligand to the cell; also reverse transmission of the intercellular signals towards the ligand takes place which in turn regulates the correlation affinity and the force of interaction [78–80]. As a result a large number of signal molecular cascades are activated which lead to structural and physiological changes in the cells responsible for supporting focused adhesion, proliferation, indirect cell cycle regulation [81]. In the process of fibrin matrix inner surface endothelization in the vessel replacing device both precursor cells and mature endothelial cells take part which circulate in the blood flow and mig-

rate from the anastomosis ends with the native vessel [82–84]. ECs express 13 types of integrins; among these the ones taking most active part in the process of adhesion with subsequent endothelization are the β1, αvβ3 и αvβ5 subfamilies [85]. An emphasis on protein ligands conjugated with certain integrin receptors and their cell adhesion sites lays the basis for the development of inner surface modification of tissue engineered vessel replacement devices made of porous artificial material and for the stimulation of accelerated endothelization. For β1-integrins the ligands will be collagen and laminin, and the binding site – the Asp-Gly-Glu-Ala (DGEA) peptide sequence; additional recognition sites for laminin are Tyr-Ile-Gly-Ser-Arg (YIGSR), Arg-Gly-Asp (RGD) and several others. The αvβ3 and αvβ5 integrins possess an affinity to fibronectin, von Willebrand factor, fibulin, osteopontin, vitronectin with RGD adhesive peptide sequence [86–88]. The RGD-peptide may be considered a general integrin binding motive. RGD's representative versatility on the ECM makes it a maximally eligible factor for surface modification of biodegradable polymer matrixes. Research is being carried out to study peptide sequences both obtained in the course of extraction from natural materials and artificially synthesized. The latter have certain advantages: the risk of immune response is decreased as well as infections related to an insufficient degree of material cleansing. Comparison of the functional properties of natural RGD-containing proteins and their artificial analogs has shown that the latter are more efficient [89].

Artificial synthesis enables to obtain various configurations of the RGD peptides which possess various potential of interacting with cells. The overall number of studied configurations can be divided into 2 groups: non-cyclic (linear) and cyclic forms. It has been shown that cyclic RGD peptides are the ones to be bound to αvβ3 integrins [90]. The ligand may be either of natural origin or synthesized (linker). Control of the specific interactions between cell receptors and ECM ligands is a critical aspect in tissue engineering as it ensures the effectiveness of cellular migration and adhesion [91]. It has been shown that it is the length of the ligand that ensures bioavailability of the RGD peptides for integrin mediated interaction with the cell and further regulation of the adhesion force and migration speed [92]. The number of 'polymer composition – linker – RGD peptide' is quite large, therefore currently the issue regarding priority RGD peptide modification of polymer vascular prostheses is open.

RGD configuration types and ligands/markers conjugated with them

Synthesized GRGDDSP peptides immobilized on a PCL graft by means of water resistant bioadhesive mussel fp-151 protein (MAP) has shown its efficiency when implanted into rabbits' carotid arteries. Such a coating improved endothelization of the MAP-RGD graft surface by means of active attraction of mature and progenitor endothelial cells which ensured monthly patency in nearly 70% of all cases [93]. An emphasis has been made in this work on the MAP linker: an artificially synthesized form produced from natural components turned out to be more biocompatible and quite simple in obtaining as compared to existing commercial samples [94].

A study performed by Cutiongco M.F.A. et al. (2015) included *in vitro* and *in vivo* comparison of the cyclic cRGD peptide form (CRRGDWLC) and the non-cyclic RGDS peptide cross-linked by means of PVA grafts (poly(vinyl alcohol) hydrogel) with the help of a linker produced by interphase polyelectrolyte complexing (IPC): fibre formation from chitosan and alginate. In the course of this study fibronectin and heparin performed the role of alternative modifying agents [95]. Viability of human umbilical vein endothelial cells (HUVEC) on polymer films with fibronectin coating, RGDS and cRGD showed a positive tendency towards improvement of cellular survival as compared to non-modified analogues. At the same time, modification with heparin was considered ineffective due to decreased adhesion and endothelial cell proliferation. In the course of haemocompatibility assessment samples modified by fibronectin were also excluded due to platelet activation. Polymer films with non-cyclic RGDS demonstrated platelet activation to a lesser degree than films with fibronectin. Samples with cyclic cRGD activated individual platelets that were only partly attached by pseudopodia, which indicated low platelet activation and presented this modification as most fitting for further *in vivo* testing [96].

In one of the studies by Samantha Noel et al. (2015) synthesized peptide sequences CGGRGD, CGGYIGSR and CGGREDV were studied which were immobilized via a polyethyleneglycol linker (PEG) on the surface of polyethylene terephthalate with the purpose of increasing the athrombogenic properties of the artificial material. The effectiveness of modified polymer film versions was evaluated by adhesion indicators as well as by HUVEC cell culture viability. The grafted REDV peptide did not improve endothelial cell adhesion while RDG peptide and YIGSR peptide significantly increased the metabolic activity of the cell culture. The authors noted that co-immobilization of RGD and YIGSR peptides improved HUVEC metabolic activity even further, which indicated synergism between two sequences [97]. Choi W.S. et al. (2016) performed surface modification of a polymer frame produced from a combination of polyurethane (PU) and elastomer (Pellethane) with heparin, adhesive GRGDS and YIGSR peptides via a PEG linker. *In vitro* experiment showed that the effect of RGD peptide on HUVEC cell culture adhesion and proliferation was somewhat higher than for YIGSR peptide or for the sample with co-immobilization of both peptides. Implantation

of non-modified grafts as well as those modified by heparin and by two adhesive peptides was performed for rabbits for a period of up to 2 months. The results of the experiment showed 71.4% patency in modified samples vs 46.2% in non-modified analogues [98].

In one of the works performed by the research group headed by Antonova L.V. (2015) and devoted to biodegradable graft modification by adhesive peptides the GRGDG configuration has been studied [99]. Surface modification was compared to PHBV/PCL grafts with incorporated VEGF. According to the results of short term and long term implantation of grafts with RGD or VEGF into the abdominal aorta of laboratory rats the authors noted no significant differences in cellularity evolution during the formation of endothelial monolayer which was functionally more mature as compared to non-modified grafts. Both types of modification proved to be sufficiently effective [100]. Further on, in 2019 the same research group presented the results of *in vitro* and *in vivo* studies where the results of modification by various configurations of RGD peptides and linkers immobilized on the surface of PHBV/PCL grafts were compared. The studied adhesive peptide sequences were as follows: non-cyclic RGDK and AhRGD, cyclic c[RGDFK] peptide. Cross-linking of the peptides with polymer materials was performed via linkers of different length and chemical content: short 1,6-hexamethylenediamine and long 4,7,10-trioxa-1,13-tridecanediamine. Same as in the study performed by Cutiongco M.F.A. et al. (2015) the most optimal configuration was the cyclic form c[RGDFK], however the length of the linker group made a significant impact on the bioavailability of the molecule both *in vitro* and *in vivo*. Colony-forming human endothelial cell adhesion on graft samples modified by c[RGDFK] via the 4,7,10-trioxa-1,13-tridecanediamine linker exceeded that demonstrated in comparison with other RGD-modified samples. Also it has been possible to achieve a better endothelial monolayer on the inner surface of the grafts implanted to laboratory rats, as well as 100% graft permeability at different times after implantation (1 and 3 months). At the same time hemocompatible properties of such material were higher in comparison with samples modified by the same cyclic RGD peptide but cross-linked with the polymer surface by a short linker – 1,6-hexamethylenediamine [101].

CONCLUSION

A lot of attention is devoted to growth factors and adhesive peptide sequences, in particular RGD, in various areas of development based on selective binding to target cells. Biologically active molecules – VEGF, bFGF and some others integrated into the vascular prosthesis material, have shown to be efficient in *in vitro* and *in vivo* studies. Use of several growth factors for the modification of biodegradable vessel replacement items may lead to more optimal neogenesis of true blood vessel tissues at the implant site. Affinity to endothelial cells makes RGD peptides and their configurations ideal agents for surface modification of tissue engineering constructions which contact with blood and require prompt surface endothelization. The speed of spontaneous endothelization which should be inititated during implantation of a small diameter artificial blood vessel will depend directly on the RGD peptide bioavailability which may be ensured by means of a linker of a certain length.

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FIRST EXPERIENCE IN TWO SUCCESSFUL CONSECUTIVE PREGNANCIES AFTER SIMULTANEOUS LIVER-KIDNEY TRANSPLANTATION WITH RENO-PORTAL TRANSPOSITION

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The paper presents the world's first clinical case of two full-term successive pregnancies in a patient following simultaneous liver-kidney transplantation with reno-portal transposition. Both pregnancies ended with the birth of healthy children and favorable course of postpartum and long-term periods. The features of management and childbirth are highlighted. Literature review on this problem is presented.

Keywords: pregnancy, simultaneous liver-kidney transplantation, reno-portal transposition, severe portal vein stenosis, autosomal recessive polycystic kidney disease.

INTRODUCTION

At present, active development and wide interdisciplinary interaction between obstetrics, gynecology and transplantology have been promoting successful gestation not only after transplantation of individual solid organs, but also in the case of multivisceral transplantations with complex vascular reconstructions. This has ensured favorable pregnancy outcome in complex clinical situations. Highly trained transplant specialists have made it possible to perform complex simultaneous transplantations when existing vascular complications, such as total portal vein thrombosis, without high-tech vascular reconstructions, are absolute contraindications to transplantation due to the futility of restoring normal graft perfusion, impaired graft function and engraftment in the post-transplant period [1, 2]. Most researchers consider these methods as the most complicated, associated with high morbidity and risk of death in recipients in the early postoperative period [2–6]. Single cases of successfully performed similar operations with favorable long-term outcomes have been reported by world's leading transplantation centers [7]. There are no cases of gestation in patients after such interventions described in publications. The presence of a complex of transplanted organs and complex vascular reconstruction in a pregnant woman's body causes atypical course of adaptive gestational processes, high risk of pregnancy and delivery complications. It determines the need for highly skilled obstetric and gynecological care and personalized regimens for medical prevention, diagnosis and treatment of complications. In the Republic of Belarus, a patient successfully had full-term consecutive pregnancies after undergoing simultaneous liver-kidney (SLK) transplantation with reno-portal transposition. The situation was unique in that it involved managing both pregnancies to full-term and satisfactory condition of the newborns and mother in the postpartum period and beyond. Such a clinical situation, even with one pregnancy, is not described in any publications in international databases. This determines the need to highlight the principles of its management as a high-risk pregnancy with a favorable outcome, a unique situation, not found anywhere in the world.

LITERATURE REVIEW

According to a meta-analysis by D'Amico et al. – employees at the Transplantation Center, Department of General Surgery, Digestive Disease and Surgery Institute, Cleveland Clinic, Cleveland, OH, USA – published in February 2019 in Transplant International, a total of 66 patients who underwent liver transplantations combined with reno-portal transposition are currently registered worldwide. Among them are 50 (72.7%) male recipients, 15 (22.7%) women and 1 (1.5%) 14-year-old child. Reno-portal transposition was performed in 42 (63%) whole liver transplants, in 12 (18%) split liver transplants; 7.5% (5) of grafts were derived from living donor, and 4.5% (3) were domino grafts. Overall patient and graft survival were each 80%. Overall, 71% of patients developed postoperative complications, including

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ascites in 18 patients (27.2%), infectious complications in 13 patients (19.6%), transient renal dysfunction in 12 patients (18.1%), variceal hemorrhage in 2 patients (3%), bile leak/stenosis in 4 patients (6.1%), hepatic artery thrombosis in 3 patients (4.5%), early portal vein re-thrombosis in 2 patients (3%), chronic renal dysfunction in 2 patients (3%) and late (after 12 months) portal vein re-thrombosis in 1 patient (1.5%). Out of 3 patients with hepatic artery thrombosis, 2 required repeated vascular reconstruction, whereas the remaining patient required liver re-transplantation. One of the 2 patients with early portal vein thrombosis required portal angioplasty with stenting, which resulted in a satisfactory outcome, whereas the other patient died of multi-organ failure. A patient with late portal vein thrombosis required liver transplantation and died while awaiting re-transplantation as a result of multi-organ failure. Mortality was reported to be 19.6% (13 patients). Causes of death included sepsis (4 patients, 30.7%), cerebral hemorrhage (4 patients, 30.7%), hepatocellular carcinoma recurrence (2 patients, 15.4%), variceal hemorrhage (1 patient, 7.7%), and sudden cardiac arrest (1 patient, 7.7%) [1].

Since the model for end-stage liver disease (MELD) was introduced in 2002, there was been increased number of SLK transplantations [3, 4, 8–13]. However, reno-portal transposition during an SLK transplantation is described in only one source: Baker et al. – employees at the Northwestern University, Feinberg School of Medicine Department of Surgery, Division of Organ Transplantation, Chicago , USA – in their publication indicated that their experience was the only case of reno-portal transposition during an SLK transplantation [7].

In the case of our patient with SLK transplantation, the liver transplantation technique was complicated by the need for donor liver implantation against the background of critical portal vein stenosis and large spontaneous splenorenal shunts. Moreover, the use of a high-tech method for formation of reno-portal transposition was required.

Lai Q. et all. (2014), Starzl Unit of Abdominal Transplantation, University Hospitals Saint Luc, Brussels, Belgium, in a publication in World Journal of Hepatology, report that serious vascular complications such as portal vein thrombosis and critical portal vein stenosis were recently considered as an absolute contraindication for transplantation, and such patients were not operable. In recent years, due to improvements in surgical treatment methods, it has become possible to provide adequate hemodynamics in the portal vessels in patients with such vascular malformation by performing reno-portal or cavo-portal hemi-transposition [6]. Moreover, formation of a reno-portal transposition is the preferred method because it involves fewer complications if successful [1, 14]. However, these methods are considered by most researchers to be the most complicated, associated with high morbidity and risk of recipient death in the early postoperative period if there is incomplete correction of existing portal hypertension, leading to rapid hepatic graft dysfunction [2–6, 15–17]. To achieve satisfactory outcomes, patients should be referred to specialized centers, surgical strategy must be carefully planned before transplantation, high-tech vascular interventions may be required, and in some cases, individual transplantation technique that is unparalleled anywhere in the world may be required [5, 14, 18, 19].

Currently, there are international publications describing 6 pregnancies in 5 women after SLK transplantation. Not even a single pregnancy after SLK transplantation with renal portal transposition has been described. In the above cases, among the pregnancy complications, the following were indicated: fetal growth retardation in all patients, preeclampsia in two patients, premature birth zin four cases. Four patients were carrying pregnancy for the first time, one patient had 2 consecutive pregnancies with reversible renal transplant dysfunction during both pregnancies and a permanently impaired transplanted kidney function 17 months after delivery. These publications do not provide data on the condition of these women in the long-term period after delivery, as well as on the state of health and developmental characteristics of the newborns after the neonatal period [20–23].

Pregnancy in women after transplantation is always associated with increased risk of complications. Gestation after multivisceral transplantation is a more complicated and rarer situation. Complex vascular reconstruction during multivisceral transplantation during pregnancy is an unexplored situation requiring personalized management and an interdisciplinary approach. A physiological increase in the dynamics of pregnancy by almost 2 times the volume of circulating blood and the increasing effect of hormonal, neurogenic, and mechanical factors on the vascular wall lead to increased hemodynamic load on vascular anastomosis. At the same time, alternative blood flow in the vascular network of the transplanted complex, postoperative increase in the stiffness of the vascular wall at anastomosis sites, peculiarities of bile secretion, lipid, nitrogen metabolism and, in general, the functioning of the transplanted organs cause changes in the adaptation gestational processes, which increases the risk of pathological processes and complications.

Since 2008, 44 pregnant women with transplanted organs have successfully given birth at the Mother and Child Republican Centre for Applied Research [24–26]. We have presented the first pregnancy experience by this patient [27]. However, given the lack of such publications and the uniqueness of the clinical situation, and in order to highlight the therapeutic and diagnostic monitoring, as well as exchange of experience with foreign colleagues, the team of authors presents a clinical case of two successful full-term successive pregnancies in women after SLK transplantation with reno-portal transposition.

CLINICAL CASE

Patient A., born in 1985, was first diagnosed at the age of 10 months, when hepatosplenomegaly and kidney enlargement, accompanied by proteinuria and increased serum creatinine levels, were discovered during examination for pneumonia. The patient had a younger sibling sister, who had similar changes in internal organs. Although her parents and close relatives had no liver and kidney diseases, the disease was assumed to be hereditary. The family visited geneticists for consultations. In compliance with all K. Zerres criteria: 1) typical renal changes detected by imaging examination methods, 2) pathological and anatomical confirmation of the diagnosis in the patient's native siblings, 3) absence of polycystic kidney disease during ultrasound examination of the patient's parents above 30 years of age, 4) presence of clinically and histologically confirmed liver fibrosis, the patient was diagnosed with autosomal recessive polycystic kidney disease. Since this disease does not have any etiopathogenetic treatment and has a very unfavorable outcome in early childhood, despite symptomatic therapy, progressive deterioration of the patient's condition and development of end-stage of the disease led to the need for SLK transplantation. Final diagnosis at the time of inclusion in the waiting list: "Polycystic disease with liver and kidney injuries. Secondary chronic pyelonephritis, remission. End-stage chronic kidney failure, CKD 5D. Cryptogenic liver cirrhosis (Child-Pugh class B). MELD 19. Mixed portal hypertension. Splenomegaly. Hypersplenism. Grade 2 esophageal varices. Portal hypertensive gastropathy: chronic superficial gastritis and bulbitis. Ascites. Moderate symptomatic anemia".

Additional examination via CT angiography revealed critical portal vein stenosis and large spontaneous splenorenal shunts, formed against the background of portal hypertension. This anatomical feature significantly contributes to the hemodynamic characteristics of portal perfusion and requires vascular reconstruction at the hepatic graft implantation stage. This is necessary to prevent development of early graft dysfunction, which can occur against the background of changes in the volumetric blood flow through the portal vein due to stenosis and pronounced splenorenal discharge. In this case, reno-portal transposition becomes the optimal variant for vascular reconstruction (Fig. 1).

At the Minsk Applied Research Center for Surgery, Transplantology and Hematology (then known as Republican Scientific and Practical Center for Organ and Tissue Transplantation), on April 1, 2015, the patient underwent combined orthotopic liver transplantation with reno-portal transposition and heterotopic intra-abdominal kidney transplantation. The clinical characteristics of the disease and the surgical intervention performed are presented in table 1.

Good engraftment and graft functioning facilitated rapid recovery and achievement of high quality of life for the patient. They also restored fertility and the need for reproductive function. However, given the patient's genetically determined illness, the issue of gestation could only be resolved after genetic counseling. Based on the autosomal recessive type of inheritance of the

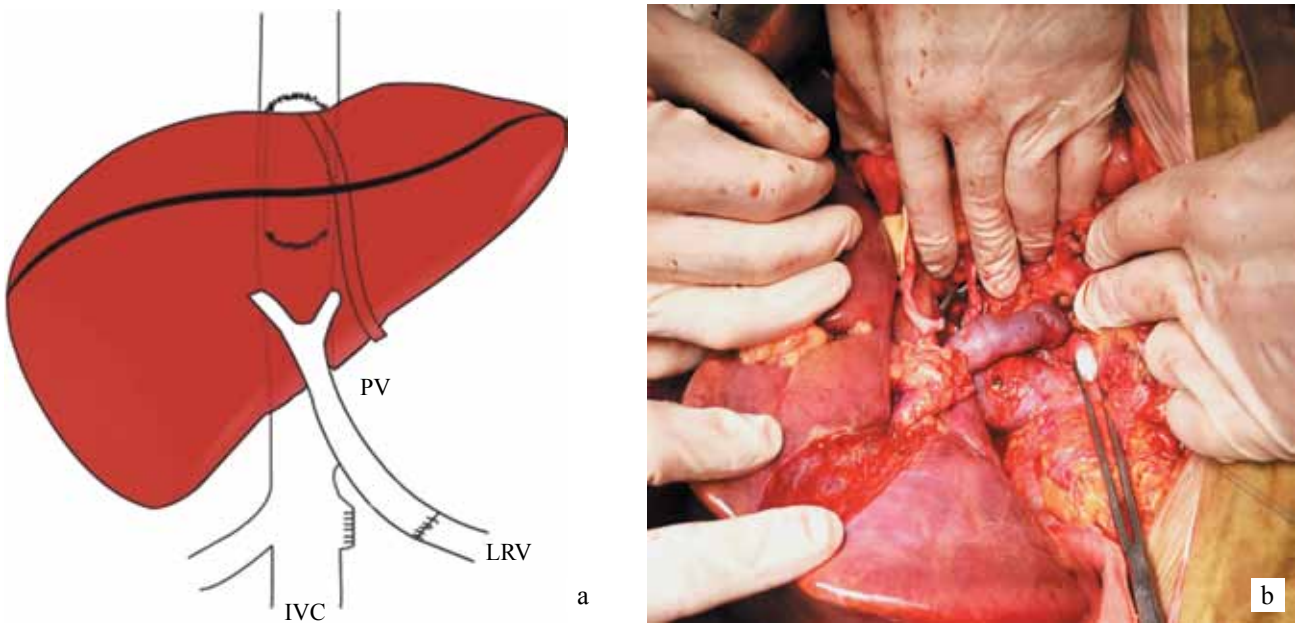

Fig. 1. Reno-portal transposition: a – graphic image of reno-portal transposition (J. P. Lerut, D. Mazza, V. VanLeeuwetal): PV – portal vein, IVC – inferior vena cava, LRV – left renal vein; b – final view of anastomosis between the portal vein of the liver graft and the recipient's (patient A) left renal vein (reno-portal transposition). Intraoperative photo from Prof. O. Rummo's archive

Table 1

Patient A's anamnestic data. Surgical intervention peculiarities

Clinical characteristics	Personalized data	
Disease that necessitated multivisceral transplantation	Autosomal recessive polycystic kidney disease (ARPKD)	
Nature of disease	Congenital, genetically determined	
Disease manifestation period	Baby age	
Age at which end-stage disease manifested	28 years	
Waitlist time	11 months	
Vascular complication, a contraindication to transplantation	Critical portal vein stenosis, large spontaneous splenorenal shunts	
Cause of contraindication	Post-transplant impaired liver graft perfusion	
High-tech vascular reconstruction that allowed for transplantation	Reno-portal transposition (see Fig. 1)	
Type of transplantation performed	Combined orthotopic liver transplantation with reno-portal transposition and heterotopic intra-abdominal kidney transplantation	
Laboratory findings before and after transplantation:	Before transplantation	After transplantation
– serum creatinine	479.2 μmol/L	74.2 μmol/L
– urea	40.0 mmol/L	4.8 mmol/L
– uric acid	595.6 μmol/L	379.2 μmol/L
– alkaline phosphatase	227.8 IU/L	49.3 IU/L
– GFR by Cockcroft–Gault	12.6 mL/min	91.4 mL/min

disease and the probability of heterozygous carriage of a mutation in a healthy husband – 1:70 (population), the risk of having a baby with autosomal recessive polycystic disease was 0.7%, which is interpreted as a low degree of genetic risk and allows planning pregnancy for this couple.

Detailed characteristics of both pregnancies, as well as regimens for prevention and treatment of complications are presented in table 2.

During both pregnancies, the woman underwent prenatal examinations according to a pregnancy screening program: combined first trimester screening (ultrasound examination of the fetus with measurement of the thickness of the collar space and determination of three biochemical markers), ultrasound examination of the fetus at 20 and 32 weeks of gestation. Prenatal examination did not detect any fetal pathology. Doppler and cardiotocographic examinations showed that the condition of the fetus remained satisfactory throughout the observation period.

Delivery in both cases was performed through caesarean section in full-term pregnancy. Indications for caesarean delivery were: anatomically narrow pelvis, condition after multivisceral transplantation with vascular reconstruction, operated uterus (in the second pregnancy). Anesthetic management method included combined spinal and epidural anaesthesia. Given the undesirability of increasing the metabolic load when prescribing hormonal contraception and the risk of infection during installation of intrauterine systems, sterilization was performed during the second operation. Based on international data indicating the safety of breastfeeding when taking tacrolimus drugs, the patient was asked to continue to breastfeed the baby. But for her own reasons, she refused. Lactation was suppressed by administration of cabergoline. The postpartum period was uneventful. Both times, the sutures were removed on day 8, and the patient was discharged home with the child on day 9.

At present, it has been 2 years 7 months since the first and 5 months since the second birth. The patient feels well. Laboratory parameters correspond to the pregestational levels. Instrumental examinations revealed no pathology. Ultrasound examination of grafts with Doppler-measuring vessels and anastomosis was performed during both pregnancies and in the postpartum period – no dysfunction was detected. The data are presented in table 3 and in Fig. 2.

The condition of both children and the neonatal period are currently fully consistent with age characteristics. Examinations found that laboratory indicators correspond to that of the population. The boy is attending a standard-type preschool, he is active, 2 years 7 months of age, 94 cm tall and weighs 14.5 kg. The girl aged 5 months is 64 cm tall and weighs 7.2 kg. Cognitive development of both children with no abnormalities.

This family will be undergoing regular medical checkup: apart from general clinical examinations, diagnostic monitoring, as during pregnancy, includes in-depth examination of the urinary and digestive systems with identification of early markers of renal and hepatic injury, tumor markers. The children will continue to be monitored in order to determine if their health and development characteristics are normal.

Table 2

Clinical characteristics of pregnancy and childbirth, prevention and treatment regimens for complications in patient A during first and second pregnancy

Clinical characteristics	First pregnancy	Second pregnancy
Pregnancy		
How long has the transplanted organ been in the body of the pregnant woman	11 months	3 years 1 month
Immunosuppressive therapy regimen	Tacrolimus (advagraf): daily dose was increased with time from 5 to 7 mg, methylprednisolone: from 2 to 4 mg	Tacrolimus (advagraf): daily dose was increased with time from 7 to 8 mg
Pregnancy complication risk groups	Preeclampsia Placental insufficiency, Graft dysfunction/rejection, Thromboembolic complications, Severe anemia of combined genesis, Gestational diabetes, Infection	
Prevention regimens (drug groups)	Anticoagulant therapy (drugs with angio-protective effect in prophylactic doses); Hepatoprotective agents: ursodeoxycholic acid, essential phospholipids; Metabolic agents, amino acids and their derivatives: ademetionine; Agents for treating liver diseases: artichoke leaf extract; Phytopreparations for prevention of urinary tract infections in obstetrics; Iron/folic acid/vitamin b drugs	
Pregnancy complications	Anemia of combined genesis, Threatened miscarriage in the 18–19-week period, Vaginitis	Anemia of combined genesis, Placenta previa, Hyperfermentemia Asymptomatic bacteriuria
Treatment regimens (drug groups)	Combined antianemic agents of ferrous iron with folic, ascorbic acid, cyanocobalamin, parenteral administration of iron/vitamin B drugs; Antispasmodic therapy: drotaverine / papaverine; Vaginal suppositories with metronidazole and miconazole for vaginal sanitation	Parenteral administration of iron/vitamin B drugs; with decreased iron metabolism, hemoglobin concentration <90 g/L – subcutaneous injection of erythropoietin; Hepatoprotective agents: ursodeoxycholic acid 250–500 mg/day, essential phospholipids 1800 mg/day, Metabolic agents, amino acids and their derivatives: ademetionine Fosfomycin, phytopreparations for prevention of urinary tract infections
Delivery time	38–39 weeks (268 days)	37–38 weeks (262 days)
Condition of the newborn and postpartum period		
Sex of newborn	male	female
Weight, g	3030	2790
Height, cm	47	50
Apgar score	8/9	8/8
Condition of newborn	Satisfactory	Satisfactory
Complications in newborn	–	–
Complications in mother	–	–
Rooming-in	+	+
Discharged home with the mother / transferred	Discharged	Discharged
Discharged on day ...	9	9

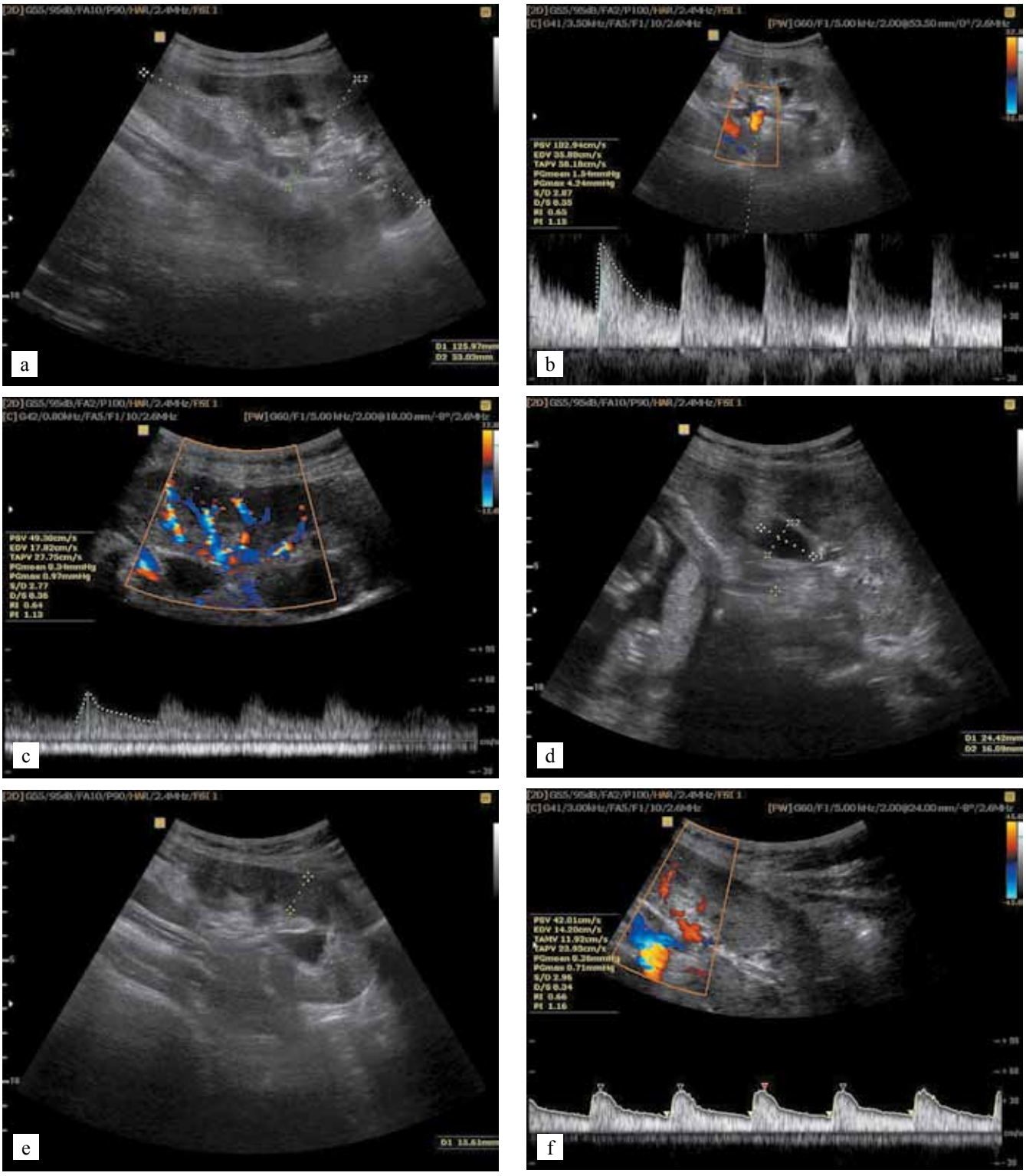

Fig. 2. Ultrasonic characteristics of transplanted complex on day 8 of the postpartum period. Transabdominal ultrasound with a 3.5–5 MHz transducer in the "abdomen" menu: 1–3 – ultrasound image of the renal graft: a – B-mode, b – Doppler ultrasound of blood flow in the main trunk of the graft renal artery, c – in the interlobar arteries of the renal graft; d–f – ultrasound image of the liver transplant gate structures: d – *vena portae*, B-mode, e – reno-portal anastomosis, B-mode, f – Doppler ultrasound of blood flow in *vena portae* and reno-portal anastomosis

Table 3

Laboratory parameters for both pregnancies and after delivery

Parameters	Before pregnancy	1st pregnancy				After the 1st pregnancy (after 1 year)	2nd pregnancy				After 2nd pregnancy (after 5 months)
		1 trimester	2 trimester	3 trimester	Postpartum period		1 trimester	2 trimester	3 trimester	Postpartum period	
Creatinine, μmol/L	67.5	91.2	72.0	98.0	93.0	70.9	87.0	71.0	127.0	97.0	69.7
Urea, mmol/L	4.7	5.0	4.8	6.4	6.2	5.7	5.3	5.8	8.0	6.5	5.3
Alt, IU/L	19.0	15.1	18.1	17.0	15.0	9.0	8.0	9.0	35.1	18.0	19.0
Ast, IU/L	10.8	11.3	15.9	26.0	24.1	12.0	14.0	10.2	38.0	27.2	16.0
Hgb, g/l	118.0	102.1	96.4	92.9	104.3	108.2	107.0	92.8	92.4	103.0	114.0
Platelets, 109/L	79.6	67.1	102.0	129.1	185.0	72.4	82.5	87.3	95.6	119.0	91.4
SPB, g/day	0	0.06	0.08	0.13	0.03	0	0.022	0.06	0.125	0	0
GFR by Cockcroft–Gault, mL/min	94.0	88.2	74.1	57.4	89.3	97.0	87.6	88.1	86.4	92.3	93.0

CONCLUSION

This case from practice represents a unique clinical situation where, despite the high risk of complications, a patient with multivisceral transplantation and high-tech vascular reconstruction, was able to endure two full-term consecutive pregnancies that ended in the birth of healthy babies. There was a favorable course of postpartum and long-term periods, reaching the pre-test level of laboratory indicators. There were no pathological changes in the morphological and functional characteristics of the formed complex. Due to absence of similar observations in international medical literature, we conclude that this is the first world experience of pregnancy in a patient with such diagnosis.

The authors declare no conflict of interest.

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SIMULTANEOUS LAPAROSCOPIC BILATERAL NEPHROURETERECTOMY, CADAVERIC KIDNEY ALLOTRANSPLANTATION AND PERFORMANCE OF VESICOSTOMY IN A PATIENT WITH NEUROGENIC BLADDER

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We present a case of simultaneous laparoscopic bilateral nephroureterectomy, cadaveric kidney allotransplantation and performance of vesicostomy. This observation shows that patients with end-stage kidney disease, primarily caused by neurogenic bladder dysfunction, can be successfully treated via surgery. The course of early postoperative period and further rehabilitation did not differ significantly from that obtainable after standard kidney allotransplantation.

Keywords: kidney allotransplantation, vesicostomy, neurogenic bladder, chronic kidney disease, laparoscopic nephrectomy, immunosuppression.

INTRODUCTION

Neurogenic bladder dysfunction comprises a group of the bladder function disorders that occur in diseases of brain and spinal cord, as well as peripheral nerves and intramural plexuses. Many urinary dysfunctions, in particular those that can damage the upper parts of the urinary tract, are centered around the lack of coordination of the activities of detrusor, bladder neck or external sphincter. These disorders occur either separately or in combination and often cause an increase in intravesical pressure without obvious neurological underlying pathological processes [1]. Idiopathic hyperactive bladder (HAB) is a quite common syndrome with symptoms of urgency, increased urination and, in some patients, urinary incontinence. The clinical manifestations of HAB are not as dramatic as the manifestations of a neurogenic bladder.

The problem background is featured by the high prevalence: about 11 million people in Russia would notice similar symptoms [2]. There are many reasons for the lower urinary tract dysfunction (LUTD), which can be generally classified as congenital structural abnormalities (posterior urethral valve, vesicoureteral reflux and Eagle–Barrett syndrome), neurological disorders (spina bifida, pathological changes at the level of the basal ganglia of the brain, spinal canal stenosis, peripheral neuropathy, etc.) and those caused by neurological pathology (diabetes mellitus, bladder tuberculosis, benign prostatic hyperplasia, prostate cancer, retroperitoneal fibrosis, urolithiasis, etc.).

In patients with neural tube defects, the risk of dysfunction of the lower urinary tract which entailed renal failure is eight times higher, and in patients with paraplegia / tetraplegia this risk is five times higher compared to the general population. Neurogenic conditions compromise the safe, effective, and controlled urine retention and the urination process. Constant high intravesical pressure is the predominant factor in kidney damage. Intravesical pressure exceeding 35–40 cm H_2O is accompanied by vesicoureteral reflux (VUR), dilatation of the upper urinary tract and pyelorenal reflux leading to nephropathy.

Urinary tract infections and nephrolithiasis are additional damaging factors. Indications for surgical correction of the lower urinary tract are determined individually, with the aim of creating a urinary reservoir with low pressure and adequate function. Surgical treatment options include continent appendicostomy (Mitrofanoff stoma), augmentation cystoplasty, ileal conduit (Bricker operation) or ureterocystoneostomy. Despite surgery, many patients will eventually reach the terminal stage of chronic renal failure (CRF), thus needing renal replacement therapy and transplantation. A high incidence of bladder dysfunction in varying degrees is observed in patients with chronic kidney disease (CKD).

In this group of patients, the major problems are bladder hyperactivity, detrusor instability, and detrusor-sphincter dysinergy. Patients with end-stage renal failure are usually oligo- and anuric and more often do not have complaints against this background, although may still feel discomfort in the abdominal cavity and micturition urgency [3]. With a small bladder capacity and hyperactive symptoms, about a quarter of patients

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with end-stage renal disease (ESRD) showed moderate severity of IPSS – 21.3% of women and 26.6% of men, regardless of hemodialysis [4]. In Chen J.L. abnormal accumulation function was observed in 71% of ESRD patients, and obstructive in 51.6%, as well as chronic inflammation and urothelial dysfunction in 48.4% of patients with cystoscopy [5].

Silva D.M. observed vesicouotetral reflux 110 of 622 (17.5%) patients, and residual urine in 83 (13.6%), respectively [6]. Bladder augmentation or diversion is the only option for kidney transplantation in recipients with NBD, and satisfactory results of this surgical intervention were obtained in comparison with the general population of recipients with normal NMP function [7–9]. The patients with bladder dysfunction were widely believed not to be considered as candidates for kidney transplantation.

There are publications with the results showing no difference in the graft survival rates and patient survival [10–12]. In controlled trials, patients with lower urinary dysfunction had a mortality and survival coefficient similar to the control group without bladder dysfunction; however, there is a high risk of MVP infection, which in turn is accompanied by minimization of immunosuppressive therapy and may lead to an unfavorable outcomes [13–16]. Renal transplant recipients with LUTD in history require special surgical techniques to form the urinary tract and ensure adequate outflow of urine which can be performed as a preliminary step or immediately with the kidney transplant. Depending on the specific situation, preparation may include creating an intestinal reservoir [17], a urinary subcutaneous fistula [18], and enlarging (augmenting) own bladder with an insert from the intestine or ureter [19]. Depending on the type of urine diversion, it is possible to use self-catheterization or a urine bag. Vesicostomy is considered more preferable. The vesicostomy techniques proposed by Blocksom (1958) and Lapides J (1961), in essence, consist in the formation of a vesicocutaneous fistula and today are widely used abroad [20] The vesicostomy disadvantages are complications rating from 5 to 20% [21]. Typical complications of the Blocksom technique are the bladder mucosa prolapse observed in 6–15% of patients, and stenosis of the vesicocutaneous anastomosis which occurs in 15–22% of cases. The disadvantages in the methodology of these techniques are the bladder capacity decrease and chronic cystitis. This category of patients is under a high risk of developing the urinary tract infections which requires appropriate prevention and / or treatment.

CLINICAL CASE

Patient, 22, diagnosis: abnormal urinary tract development, bilateral vesicoureteral reflux. Chronic pyelonephritis, latent course. (GFR 16 ml/min/1.73 m^2). CKD 4. Neurogenic bladder dysfunction. Detrusor-sphincter dyssynergia.

Anamnesis: at the age of 4, the first symptoms of the disease: febrile fever, acute urinary retention. 2000, at the same age: vesicoureteral reflux on the left. Surgery: plastic of the intramural part of the left ureter with antireflux protection. Irregular visits to a nephrologist, CKD gradually progressed. 2017: sent to the Research Institute of Urology of the National Medical Research Center of Radiology of the Ministry of Health of Russia to discuss the possibility of kidney allotransplantation.

Ultrasound: the shrunken kidney on the right, the dilated calices-pelvis system (CPS): calyx 0.6 cm, pelvis 1.3 cm, the upper ureter is not expanded. The left kidney: 60.0 cc volume, parenchyma – 1.4–0.9 cm. Dilated CPS: calices 0.8 cm, pelvis 1.7 cm, the upper ureter expanded to 0.4 cm. The bladder: 600 ml volume, distinct scalloped contours, diffusely thickened walls (1.0 cm).

Cystography (Fig. 1): contrast, 870 ml, no urge to urinate, the tower-shaped bladder with fuzzy, uneven contours. VUR right III–IV.

Complex urodynamics (Fig. 2): high-amplitude detrusor hyperactivity. Reduced bladder sensitivity, urination is not fixed on 800 ml.

Subsequently, an arteriovenous fistula of the left forearm was formed, and renal replacement therapy with programmed hemodialysis started. The patient is on the waiting list for the donor kidney.

2017: Research Institute of Urology, National Medical Research Center of Radiology of the Ministry of Health of Russia, laparoscopic bilateral nephrurethe-

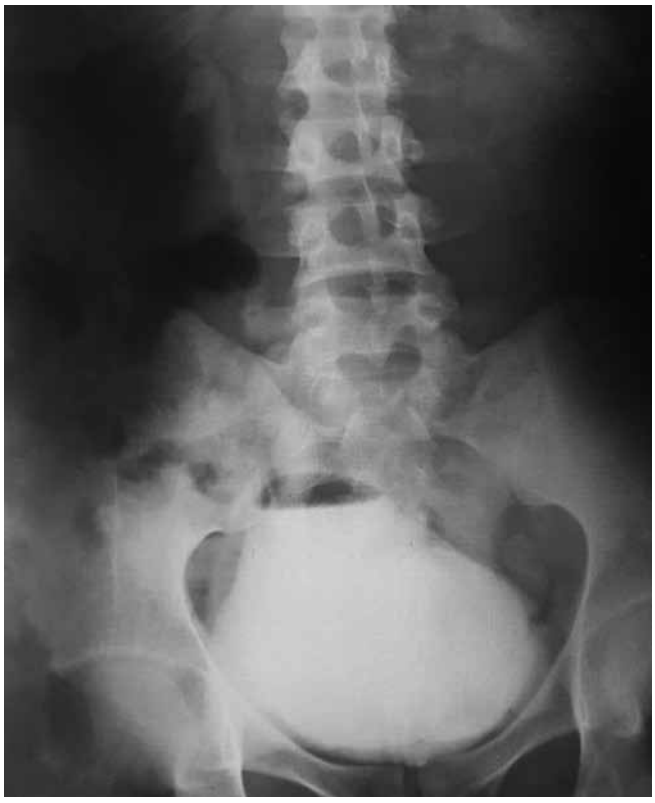

Fig. 1. The cystoradiogram

rectomy, allotransplantation of a cadaver kidney on the right, formation of continental vesicostomy. Final stage of the operation, see Figs. 3. and 4.

Renal transplant ischemia – 11 h. Total surgery – 5 h 45 min.

Stable postoperative period. The primary function of the transplant. Standard immunosuppressive therapy. The postoperative drainage and urethral catheter were removed on the 2nd day after surgery, the vesicostomy catheter was removed on the 18th day after surgery with its prior clamping. Three weeks later, the patient began to self-catheterize after each urination. On the 26th day after surgery: vesicostomoscopy, cystoscopy, the internal stent of the transplanted kidney removed. Bladder trabecularity III – pronounced bladder dysfunction.

Control ultrasound of the graft: no CPS dilatation.

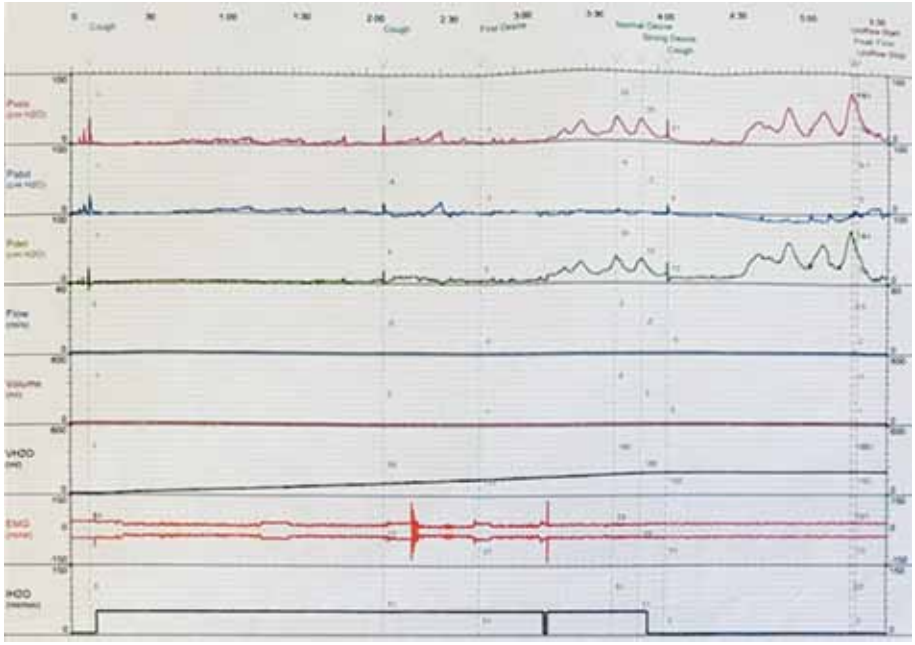

Fig. 2. Complex urodynamic examination

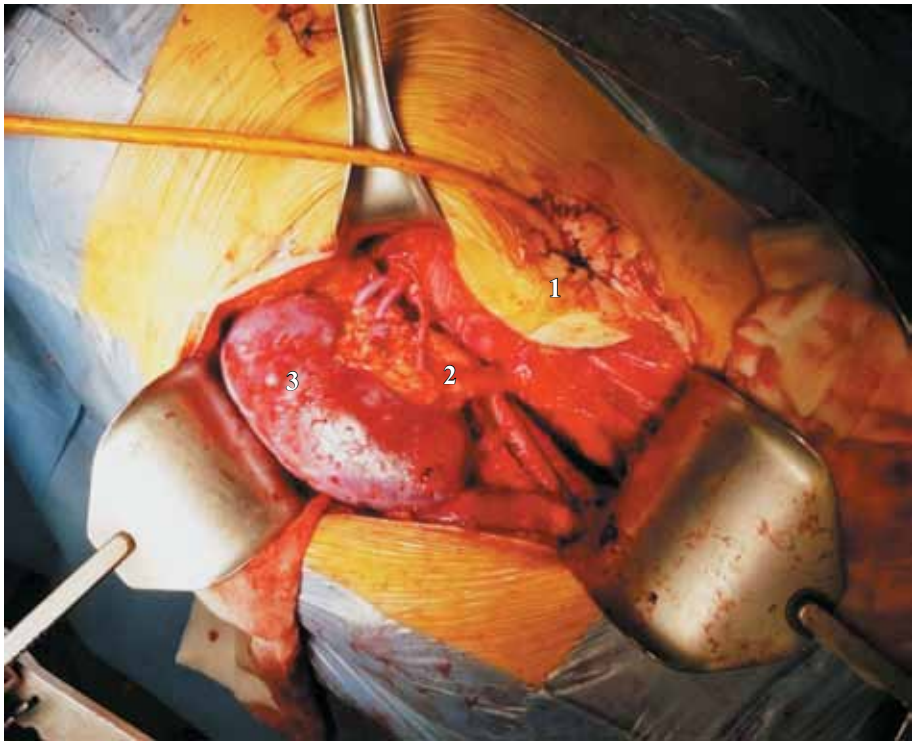

Fig. 3. Surgical field at the final stage: 1 – the excluded vesicostoma on the anterior abdominal wall; 2 – ureter of the kidney transplant; 3 – kidney (transplantate)

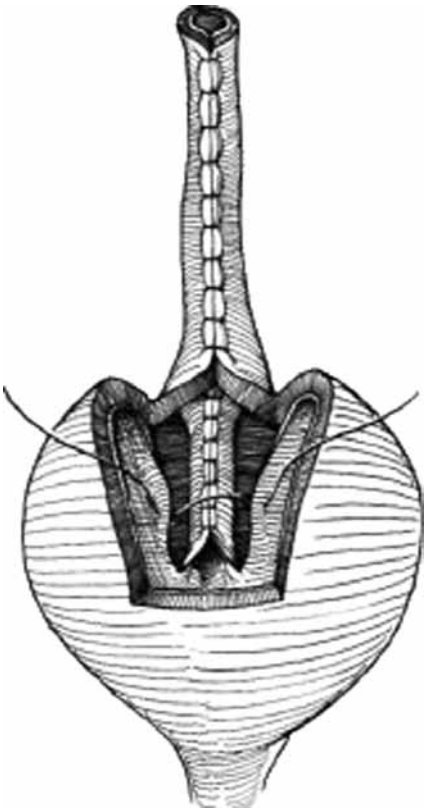

Fig. 4. The scheme of formation of a vesicostomy

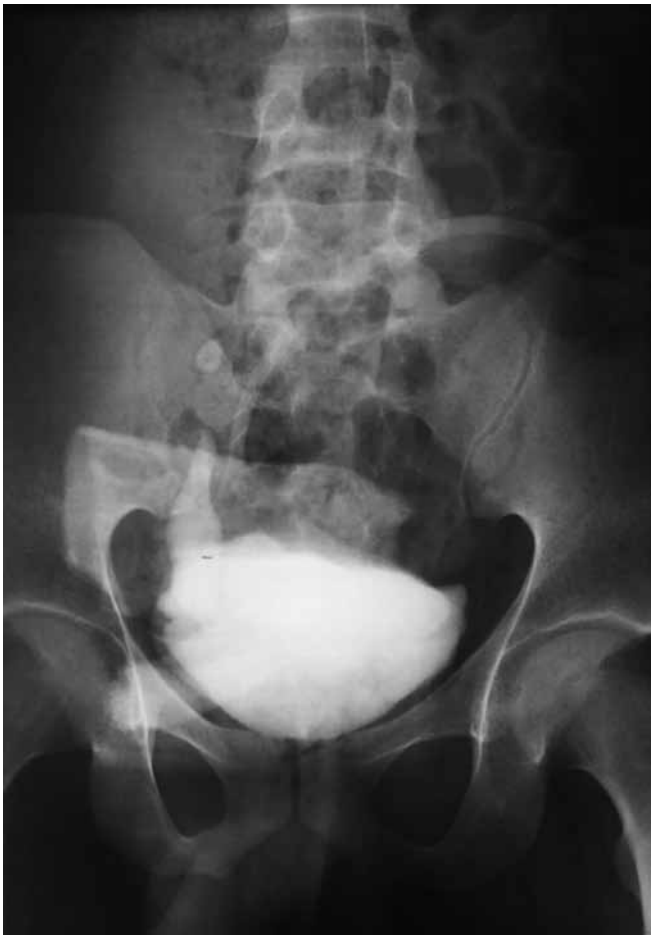

Fig. 5. The cystoradiogram after surgery

Follow-up, 19 months: monthly outpatient observation, urination with catheterization through vesicostomy (6 times a day), spontaneous urination. One episode of acute urinary tract infection.

Laboratory analyses at the time of the last observation:

CBC: Hb 120 g/dl, RBC 3.2 × 10^{12}/l, PLT 147 × 10^{9}/l, ESR 4 mm/h. Blood biochemistry: creatinine 115 μmol/l, urea 6 mmol/l, K 4.3 mmol/l, Na 144 mmol/l, Ca 1.2 mmol/l. UA: specific gravity 1,016, no protein, RBC 0–1 /hpf, WBC 0–1 /hpf.

Transplanted kidney ultrasound: 12.1 × 5.3 × 5.3 cm, up to 180 cm^3 volume; parenchyma thickness of up to 1.8 cm; adequate blood flow; R_i 0.70, CPS is not dilated, the ureter is not dilated.

To exclude vesicoureteral reflux, cystography was performed through vesicostomy. 250 ml of X-ray contrast; no vesicoureteral reflux (Fig. 5).

Urodynamics: reduced bladder sensitivity, the urge to urinate is fixed at 270 ml volume.

DISCUSSION

The described case of simultaneous laparoscopic bilateral nephroureterectomy, cadaver kidney allotransplantation, and performance of vesicostomy proves the successful simultaneous complex surgery with the subsequent satisfactory rehabilitation.

The decisive role in the simultaneous surgical intervention was played by a stable predialysis condition, intact renal excretory function of the kidneys which ensured constant bladder filling, which in turn contributed to the formation of vesicostomy with good volumetric capacity and functionality. At the terminal stage of chronic renal failure, hemodynamic, excretory, and endocrine functions are supported. The conclusion in numerous studies is that bilateral nephrectomy, as a separate type of surgical intervention before kidney transplantation, is featured by a significant risk of mortality and morbidity, so it is not the method of choice in LUTD patients with high bladder capacity [22, 23]. At the same time, simultaneous kidney transplantation with bilateral nephrectomy has a higher level of urological complications, blood loss and the need for blood transfusion than bilateral nephrectomy as the first stage [24, 25].

Bladder dysfunction can provoke a urinary tract infection which can affect renal transplant survival. Thus, close attention must be paid to the symptoms of LUT dysfunction after a successful kidney transplant. Few studies have studied lower urinary tract dysfunction and lower urinary tract symptoms (LUTS) in recipients. Experimental and clinical studies have repeatedly shown that the restoration of diuresis leads to manifestations of LUTS and urinary dysfunction. When this happens, repeated urinary infections or other pathological changes in the bladder can lead to structural rearrangement of all LUT layers and serious morphological and functional

abnormalities, such as a bladder contraction with minimal capacity. After successful kidney transplantation, dysfunction and LUTS can be detected along with all their negative consequences. Anuria and oliguria are the most important risk factors for urological complications after kidney transplantation [26, 27]. The transplant team should be wary of possible sources of infection [28, 29]. In patients with neurogenic dysfunction, an adequate form of urine derivation should be performed. The stages of assistance and the types of derivation of urine should be determined individually [30]. The choice of the formation of a vesicostomy in a patient consisted of a large bladder with a volume of up to 1000 ml, which made it possible to freely form a stoma from the tissue of the bladder. In the early and late postoperative periods, the vesicostomy carried out and ensures complete drainage of the bladder, which is the prevention of vesicoureteral reflux into a transplanted kidney, which in turn can lead to transplant reflux nephropathy or the urosepsis development.

CONCLUSION

At this time, there are no clear recommendations for renal transplant recipients. It is necessary to screen for LUTD symptoms, since this pathology can cause vesicoureteral reflux, induce urinary tract infections, and further complications associated with the above. Comprehensive urodynamics assessment before kidney transplantation is important as a mandatory examination of recipients as it will reveal existing dysfunctional disorders of urination and / or bladder, which will help to avoid further allograft dysfunction. Urodynamic examination after transplantation is necessary depending on the degree of dysfunction.

Simultaneous laparoscopic bilateral nephroureterectomy, cadaver kidney allotransplantation, and the formation of vesicostomy can be the operation of choice in patients with ureterohydronephrosis of their kidneys, neurogenic bladder dysfunction, leading to terminal renal failure.

The authors declare no conflict of interest.

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FATAL PROGRESSION OF SQUAMOUS CELL CARCINOMA 10 YEARS AFTER CADAVERIC KIDNEY TRANSPLANTATION

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Various research has shown that non-melanocytic malignant skin lesion is one of the most common post-kidney transplant neoplasms. Multiple lesions and a more aggressive clinical course are more common in kidney transplant patients than in the general population. This paper presents a case of malignant skin neoplasms in a patient 10 years after cadaveric kidney transplantation. The patient received standard 3-component immunosuppression with satisfactory graft function (serum creatinine level remained at 157–178 μmol/L). Scalp neoplasm was removed. Histological examination revealed a morphological picture characteristic of basal cell carcinoma with squamous differentiation. Subsequently, a relapse of the skin neoplasm of the temporal region, as well as new lesions in the frontal region and the skin of the anterior chest wall, were discovered. Despite surgical treatment and close-focus x-ray radiation, the disease rapidly progressed and eventually led to death. Squamous cell carcinoma can progress very rapidly in patients after solid organ transplantation, despite ongoing combination treatment. Perhaps in such cases, it is worth cancelling immunosuppressive therapy completely and removing the kidney graft in order to control progression of the malignant tumor process.

Keywords: skin neoplasms, squamous cell carcinoma, kidney transplantation, immunosuppression.

INTRODUCTION

After solid organ transplantation and long-term immunosuppressive therapy, patients have a higher risk of developing skin infectious and oncological complications.

There are reports confirming the risk of developing skin cancer in this group of patients [6]. It is noted that increased risk of skin cancer in kidney-transplant recipients receiving immunosuppressive therapy is proportional to therapy duration and dosage. In addition to immunosuppressive therapy, other risk factors of skin cancer include exposure to ultraviolet (UV) radiation, childhood sunburn episodes, presence of solar keratoses, presence of pre-transplant tumor lesions, and male.

OBSERVATION DESCRIPTION

In 1999, 37-year-old patient S., started having increased blood pressure up to 200/120 mmHg. An examination in 2002 revealed changes in the general urine analysis (proteinuria, erythrocyturia). After a follow-up examination, he was diagnosed with chronic glomerulonephritis. The patient did not visit a nephrologist. In connection with progression of chronic renal failure since the end of August 2004, renal replacement therapy by long-term hemodialysis was started.

Having secured a suitable cadaveric donor, an operation was performed in January 2005 – cadaver kidney allograft, with immediate graft function. The starting immunosuppressive therapy regimen was three-component (cyclosporine, mycophenolate mofetil, methylprednisolone) without monoclonal and polyclonal antibody induction. The postoperative period was uneventful, the patient was discharged for outpatient treatment on day 20 after surgery.

Due to development of transplant nephropathy in 2007, manifested by elevated creatinine levels to 278 μmol/L and moderate albuminuria, cyclosporin was converted to tacrolimus, with a positive clinical effect; blood plasma creatinine stabilized at 156–167 μmol/L.

By the end of 2010, the patient was hospitalized for 2-sided community-acquired pneumonia. After an antibiotic therapy course, without changing the immunosuppression regimen, he was discharged after radiographic verification of resolution of the pneumonia.

The follow-up period was uneventful; graft function was satisfactory, serum creatinine level remained at 157–178 μmol/L.

At the next visit to the clinic in 2015, skin neoplasm was detected in the left temporal region. The neoplasm was removed. Histological examination revealed a morphological picture of basal cell carcinoma (BCC) exhibiting squamous differentiation, as well as focal areas of keratin pearls showing keratinization (Fig. 1).

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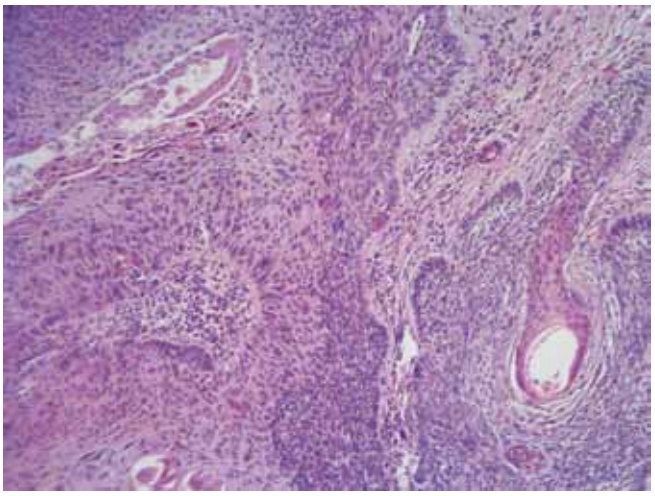

Fig. 1. Patient S. 57 years old. Morphological picture of BCC exhibiting squamous differentiation; presence of keratin pearls showing keratinization. Hematoxylin and eosin stain. ×100

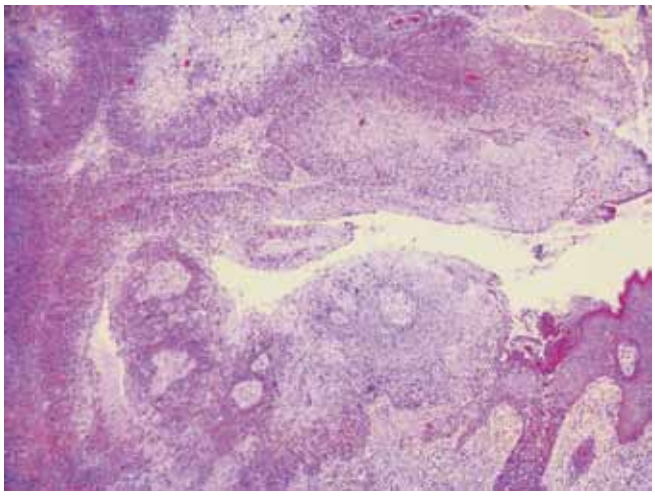

Fig. 2. Poorly differentiated squamous cell carcinoma. Hematoxylin and eosin stain. ×100

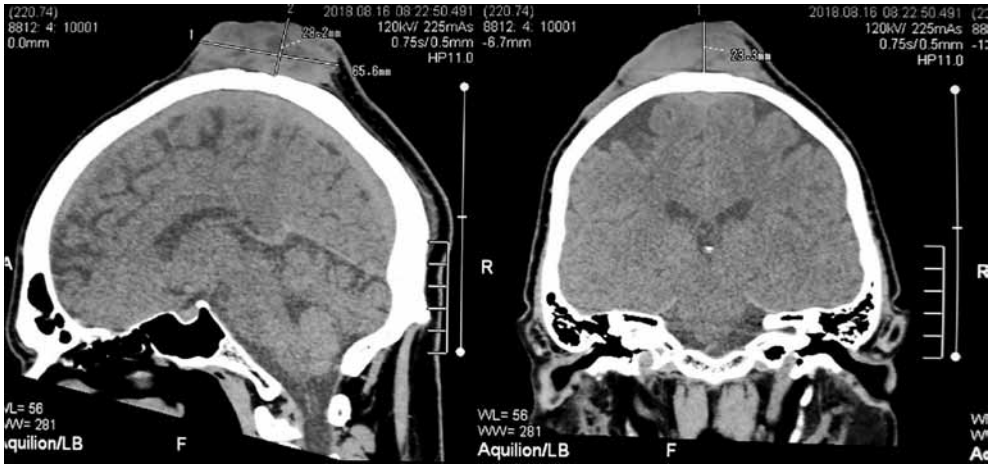

Fig. 3. CT scan of the skull. Progression of the process into the soft tissues of the parietal region with destruction of skull bones

The postoperative period was uneventful. The patient was discharged for regular medical checkup by an oncologist at the patient's place of residence.

In March 2018, temporal skin neoplasm recurred, and new foci were found in the frontal region, the anterior thoracic wall. Wide excision of all skin neoplasms from the base was performed. Histopathological examination confirmed squamous cell carcinoma (SCC) (Fig. 2).

The postoperative period was uneventful. Due to progression of oncological process, a course of close-focus x-ray radiation was carried out, dosage 3–5 g/day, course 50–80 g.

Nevertheless, in July 2018, the process progressed into the soft tissues of the parietal region, destroying the skull bones (Fig. 3, 4). A biopsy of the parietal soft-tissue tumor was performed. Histological examination revealed a poorly differentiated SCC.

Despite ongoing therapy, the disease progressed, resulting in death three years after onset of the disease.

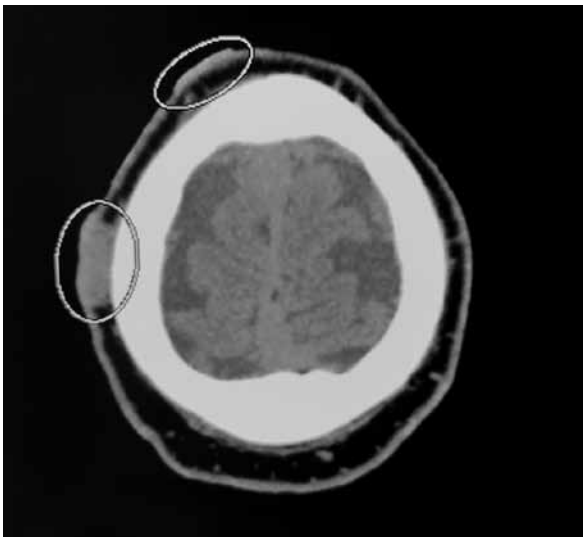

Fig. 4. CT scan of the skull. Progression of the process into the soft tissues of the parietal region with destruction of the skull bones

DISCUSSION

Numerous data indicate that non-melanocytic skin cancer is one of the most common neoplasms in kidney transplant recipients [8, 9]. Multiple lesions and a more aggressive clinical course are more common in kidney transplant recipients than in the general population [1, 2].

The risk of developing skin cancer varies by geographic region. In particular, Australia has the highest incidence.

Sun exposure is one of the main risk factors for nonmelanoma skin cancer (NMSC) in kidney recipients. So according to Joanna Sułowicz et al., of all 53 NMSC lesions diagnosed in 25 of 486 patients with a transplanted kidney, 34 (64.2%) were located on the face, which is the area most exposed to ultraviolet radiation.

In a study by Imko-Walczuk on a population of kidney transplant recipients from Gdańsk in Poland, location of skin cancers was similar to that observed in the general population (76% of SCCs and 72% of BCCs were related to the head and neck regions) [6].

The researchers found that NMSC occurrence increased with duration of immunosuppression and was 20.7% at 5 years, 37.35% at 10 years, and 53.08% at 15 years post-transplantation [5].

Most publications contain reports that SCC incidence prevails over that of BCC [4, 7].

Squamous cell skin cancer is a tumor originating from the squamous epithelium. It has a higher degree of malignancy than other skin tumors, and usually transforms from some precancerous diseases.

CONCLUSION

It is quite difficult to reliably assess the risk factors for development and progression of malignant skin tumors in our patient.

Nevertheless, the Volgograd Oblast is a region with a fairly high UV load, which, of course, can have an additional effect on patients at risk, particularly after solid organ transplantation. The first signs of the disease were noted 7 years after conversion of immunosuppressive therapy from cyclosporine to tacrolimus.

Surgical and radiation treatments for SCC of the skin on the background of immunosuppression in a transplant recipient was accompanied by rapid progression despite treatment, resulting to death. Perhaps, in cases of rapid progression of malignant skin lesions in kidney transplant recipients, complete withdrawal of immunosuppressive therapy with high chances of graft loss in an attempt to save the patient's life may be the alternative solution.

The authors declare no conflict of interest.

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LEVOSIMENDAN IN LUNG TRANSPLANT RECIPIENTS ON VA-ECMO

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Chronic heart failure is one of the most dreadful complications in the early postoperative period following lung transplantation. At the same time, the effect of using levosimendan in the early post-lung transplant period is currently insignificant and remains debatable. This paper presents a clinical case where levosimendan was successfully used in a patient with right ventricular heart failure during lung transplantation undergoing central venoarterial extracorporeal membrane oxygenation (VA-ECMO).

Keywords: levosimendan, lung transplantation, right ventricular heart failure.

INTRODUCTION

Lung transplantation (LT) is usually the final treatment option for end-stage lung disease, when other treatment methods are ineffective [1–3].

Russian Transplant Society reports that the country has only 4 LT centers [4]. As of the end of 2019, the Sklifosovsky Research Institute of Emergency Care had completed over 60 bilateral lung transplants. The early postoperative period in lung recipients can be accompanied by complications. The most common are bleeding, graft dysfunction, and failed bronchial anastomoses. Up to 20–30% of lung transplant recipients develop acute kidney injury (AKI), with indications for renal replacement therapy (RRT); 40% of the recipients develop neurocognitive disorders in the early postoperative period [5–8]. Cardiovascular disease is one of the most dreadful complications. Lung recipients often have delayed restoration of right ventricular (RV) function due to high pulmonary hypertension, as well as isolated left ventricular (LV) dysfunction with slight involvement of the right ventricle. Levosimendan is used in intensive care for managing left-sided heart failure (LSHF) and clinically significant RV dysfunction [9–11].

At the same time, the effect of levosimendan in the early post-LT period is currently insignificant and remains debatable. There are isolated cases of successful use of levosimendan.

We present a clinical case of successful use of levosimendan in right-sided heart failure (RSHF) during lung transplantation. The patient was under central venoarterial extracorporeal membrane oxygenation (VA-ECMO).

CLINICAL CASE

A 41-year-old patient diagnosed with pulmonary non-Langerhans cell histiocytosis (group 3 pulmonary hypertension) successfully underwent lung transplantation at Sklifosovsky Research Institute of Emergency Care.

Intraoperative monitoring in LT included continuous electrocardiographic (ECG) monitoring, heart rate (HR) measurement, pulse oximetry (SpO_2), invasive hemodynamic monitoring: blood pressure (systolic blood pressure, diastolic blood pressure, mean arterial pressure), central venous pressure (CVP) and pulmonary artery pressure with a Swan–Ganz catheter (pulmonary artery pressure, pulmonary wedge pressure / pulmonary capillary wedge pressure, cardiac output).

Induction anesthesia was carried out with propofol (2 mg/kg), fentanyl (4 μg/kg) and cisatracurium besylate (150 μg/kg). After preoxygenation with 100% oxygen, the patient was separately intubated with a Robert Shaw tube (No. 37). Pressure support ventilation (PSV) was set. Respiratory parameters (respiratory minute volume, respiratory volume, respiratory rate, peak inspiratory airway pressure (Ppeak), positive end expiratory pressure (PEEP), and gas exchange (FiO_2, concentration of inhaled anesthetic) in the respiratory circuit were measured using a Dräger Primus gas analyzer (Germany).

Anesthesia was maintained by intravenous injection of cisatracurium besylate (1.5 μg/kg/min) and fentanyl (100–150 μg/kg/min), as well as by inhalation anesthetic – desflurane (MAC 1.0–1.4). Depth of anesthesia was monitored using bispectral index (BIS). In an express diagnostics laboratory, analysis of oxygen status indicators (pO_2, pCO_2, ctO_2), arterial blood acid-base balance (PH, $cHCO_3$) and electrolyte blood composition (K, Na, Ca, Cl). Hemoglobin and hematocrit were also analyzed.

After anesthesia induction and at the stage of pneumonectomy on the right, hemodynamic parameters were determined to be stable (blood pressure 140–110/90–75 mm Hg). Pulmonary artery systolic pressure was

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102 mmHg. After pneumonectomy, there was a tendency to hypotension on the right (blood pressure 75–80/41–52 mmHg). In correcting the hypotension amidst higher concentration of dopamine (up to 12.78 mg/kg/min), dobutamine (5 μg/kg/min) and norepinephrine were included with gradual increase in dose to 400 ng/kg/min. With the progression of cardiotonic- and vasopressor-resistant hypotension, increase in lactate to 7 mmol/L, hypercapnia (increase in pCO_2 to 85 mmHg), central VA-ECMO was used, providing up to 3.8 L/min circulatory support on the Maquet Rotaflow pump.

The lung transplant surgery was successful. It lasted for 13 hours 20 minutes. Endoscopic examination showed that the condition of bronchial anastomoses was satisfactory. Intraoperative blood loss was 2000 mL. The patient was transferred to ICU under VA-ECMO, providing 4.0 L/min circulatory support.

In the intensive care unit, significant increase in cardiotonic and vasopressor doses was required in order to stabilize blood pressure. So, norepinephrine was used at 950 ng/kg/min, dobutamine – 14 μg/kg/min, and dopamine – 13 μg/kg/min. In addition, adrenaline was added to the therapy at 200 ng/kg/min. SvO_2 was recorded at 55–65 level during online monitoring on a Cardiohelp apparatus (Maquet). Blood pressure was 80–90/45–55 mmHg amid high levels of cardiotonic agent, ECMO flow rate increased to 4.5 L/min.

On the next day (second day of the postoperative period), continuous infusion of levosimendan at the rate of 0.1 μg/kg/min was added to drug therapy. Positive dynamics were noted already by the 12th hour after levosimendan administration had started. So, dobutamine and norepinephrine doses were reduced to 6 μg/kg/min and 150 ng/kg/min, respectively, dopamine and adrenaline were withdrawn. Blood pressure was 132–128/81–75 mmHg. After completion of 24-hour continuous infusion of levosimendan, blood pressure was in the normal range (134–123/67–73 mm Hg) without vasopressor and cardiotonic support.

On the third postoperative day in the operating room, vascular decannulation was performed as the patient was weaned off VA-ECMO. Further postoperative management of the patient was done with no significant complications. The patient was discharged on day 30.

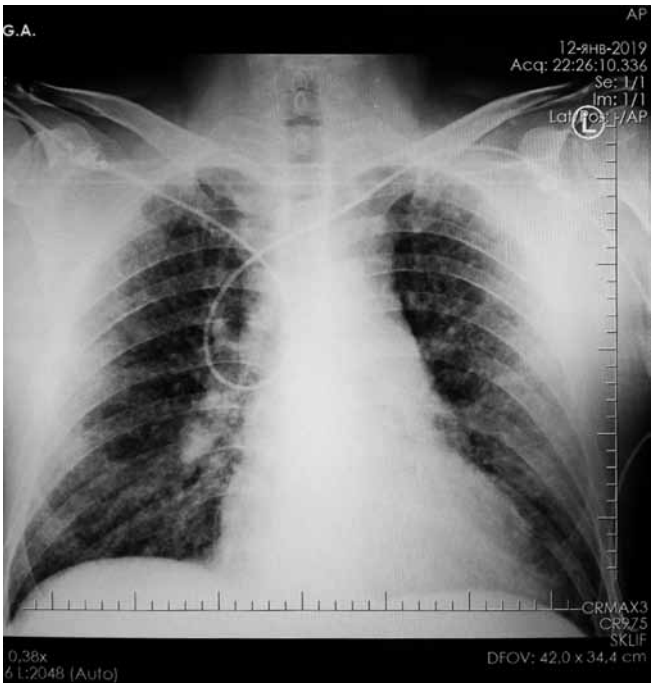

Fig. 1. X-ray picture before lung transplantation

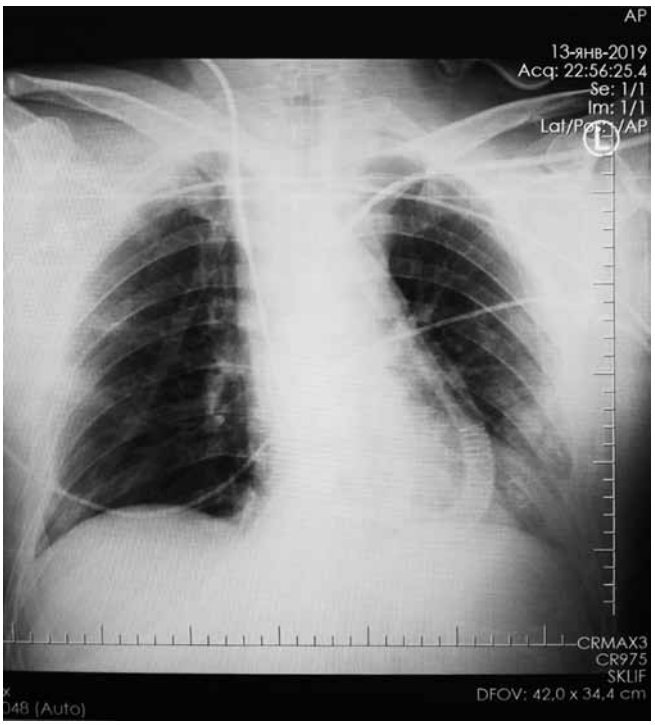

Fig. 2. X-ray picture after lung transplantation

DISCUSSION

Severe pulmonary hypertension is a risk factor for right ventricular failure in the early postoperative period after bilateral LT. Factors contributing to the development and persistence of pancreatic insufficiency are eliminated through a comprehensive approach – the use of maintenance therapy that increases cardiac output, corrects blood pressure correction, optimizes infusion therapy, reduces pancreatic afterload, prevents and treats arrhythmias and infectious complications, as well as the use of aggressive treatment methods, including continued VA-ECMO in the postoperative period [12, 13].

The mechanism of action of levosimendan is based on increased tropism for cardiomyocytes to calcium. The ability of myocyte to reduce begins with a change in cardiac troponin C configuration under the influence of calcium ions. At the beginning of systole, levosimendan is selectively bound by calcium-saturated cardiac

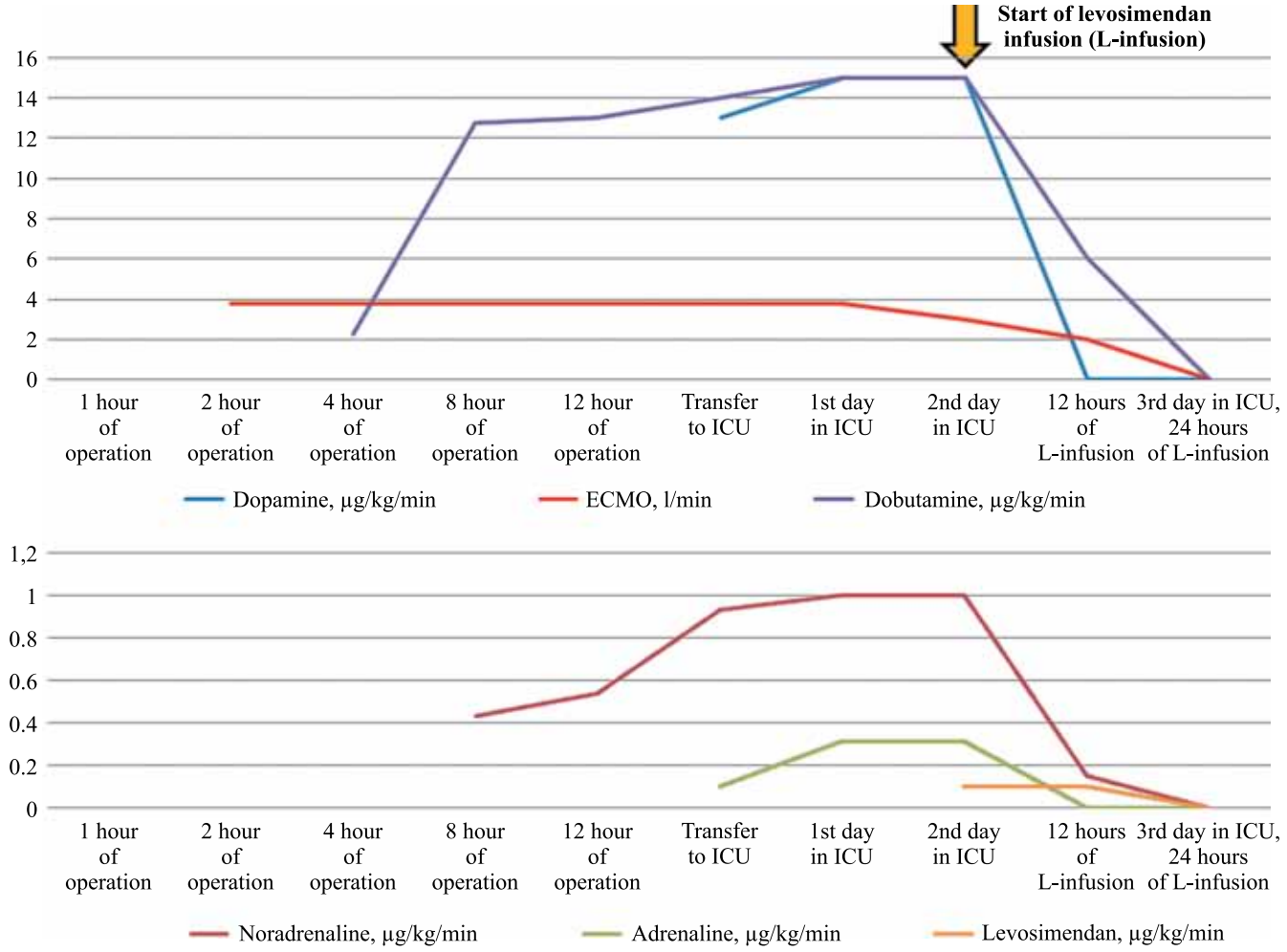

Fig. 3. Dosage of cardiotonic and inotropic agents in the early postoperative period

troponin C, which leads to stabilization of the conformation of this protein, triggering contraction of myofibrils. As a result of this interaction, connection of transverse myosin bridges with actin is lengthened, which leads to both increased strength of muscle contraction and increased number of bonds per unit time. It should be noted that the effect of levosimendan is reversible. So, in diastole at lower concentrations of calcium, the drug "frees" troponin C. This creates persistence of myocardial relaxation [14].

In an experiment on healthy animals, the ability of levosimendan to increase right ventricular contractility without significant effect on pulmonary vascular resistance was demonstrated [15]. One more mechanism of action of levosimendan by which ATP-sensitive potassium channels in the smooth muscles of the vascular wall and mitochondria can be opened should be noted. The clinical result is coronary artery dilation and reduced pulmonary blood pressure [16].

Thus, in our opinion, reduced afterload due to dilated pulmonary arteries in combination with adequate preload and cardiotonic effect of levosimendan promoted gradual cardiac output restoration and blood pressure normalization.

CONCLUSION

A 24-hour continuous infusion of levosimendan led to hemodynamic stabilization without arrhythmia induction. This agent can be used in liver transplant recipients with postoperative right ventricular failure and pulmonary arterial hypertension to significantly reduce pulmonary artery systolic pressure and increase the stroke volume.

The cardioprotective properties of levosimendan in combination with VA-ECMO, vasopressor support and optimized infusion therapy, made it possible to compensate for chronic heart failure and pulmonary hypertension in the early postoperative period.

The authors declare no conflict of interest.

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DECELLULARIZATION OF DONOR PANCREATIC FRAGMENT TO OBTAIN A TISSUE-SPECIFIC MATRIX SCAFFOLD

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One of the pressing issues in tissue engineering is on how to obtain an artificial matrix that can simulate a biological microenvironment for cells. When creating a bioengineered pancreatic construct, a tissue-specific scaffold obtained from decellularized pancreatic tissue can serve as such matrix. **Objective:** to obtain and study the characteristic properties of a tissue-specific pancreas scaffold from decellularized human pancreatic fragments. **Materials and methods.** The decellularization protocol included 3 freeze/thaw cycles, followed by treatment with surfactants (sodium dodecyl sulfate and Triton X100). At each decellularization stage, samples were routinely stained with hematoxylin and eosin and for total collagen. In addition, immunohistochemical staining of decellularized human pancreas (DHP) for type I collagen and elastic fibers was performed. Cell nuclei in the original samples and the resulting matrix were visualized using DAPI fluorescent staining. DNA quantity in the native and decellularized pancreatic tissue was determined. The cytotoxicity of the tissue-specific matrix was evaluated *in vitro* by direct contact. The matrix properties of DHP samples were determined using mesenchymal stem cells (MSCs) of human adipose tissue. **Results.** A pancreatic decellularization method is proposed. This method allows to obtain a tissue-specific matrix in the form of a connective tissue scaffold completely free of detritus with preserved thin-fiber mesh-like structure, in which elastic and collagen fibers, including type I collagen, are identified. DAPI staining confirmed the absence of nuclear material in the decellularized matrix, while residual amount of DNA did not exceed 0.1%. Absence of matrix cytotoxicity and its ability to maintain adhesion and proliferation of human adipose tissue-derived MSCs was proved. **Conclusion.** As one of the stages in creating a bioengineered pancreatic construct, a method has been developed for producing a biocompatible (lack of cytotoxicity and immunogenicity) tissue-specific scaffold from decellularized human pancreatic tissue. In the scaffold, the morphofunctional properties of the native extracellular matrix-based scaffolds of the pancreas are preserved. Adhesion and proliferation of cell cultures are ensured.

Keywords: pancreas, decellularization, tissue-specific scaffold, tissue engineering.

INTRODUCTION

Insulin-dependent diabetes mellitus is a chronic disease resulting from the depletion of a population of β-cells due to autoimmune damage. Current research aims to replenish the population of β-cells by creating bioengineered equivalents of the pancreas consisting of β-cells, stem cells or isolated Langerhans islets and a carrier matrix that ensures longer survival and efficient functioning of the transplanted cells. The materials of various nature are used as matrices for their specific physicomechanical, biological, and functional properties, such as biocompatibility, lack of immunogenicity, mechanical strength and elasticity, biodegradability, etc. [1]. The imitation of signals from the native microenvironment, i.e. tissue specificity is an important property of matrices for tissue engineering.

There is no doubt that the "native" matrix with the characteristic features of structure and composition is the most suitable for cells. To obtain a tissue-specific matrix, organs and tissues are decellularized to remove DNA, cellular material and cellular surface antigens through various methods with chemical, enzymatic or mechanical treatment [2]. Decellularization protocols should be developed considering such factors as density and thickness of the initial tissue, cell number, and lipid content [2, 3].

Extracellular matrix (ECM) is a polypeptide chain of collagen, laminin, fibronectin, and elastin intertwined with polysaccharide chains, glycosaminoglycans [4]. Typically, collagens provide the structural rigidity and tissue adhesion, supporting the integrity and shape, structure of the organ, while elastin provides strength, elasticity, and extensibility of tissue. Besides, fibronectin, fibrillin, and laminin are involved in remodeling the cytoskeleton, contractility, and differential cell adhesion [4].

The composition and organization of ECMs varies from tissue to tissue, but the main function of all ECMs

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is to provide mechanical support for the cells and maintain a number of such biological functions as cell viability and growth. In the pancreas, ECM, which contains collagen type I, III, IV, V, and VI, elastin, laminin, and fibronectin regulates the main aspects of islets biology, including development, morphology and differentiation, intracellular signal transmission, gene expression, adhesion and migration, proliferation, secretion, and survival [4, 5]. The cell-matrix interactions are important in order for mature β-cells to remain functional and avoid apoptosis, as well as to maintain the functional mass of β-cells [6]. During the isolation process, the islets often lose a substantial portion of ECM and the vascular network, subsequently negatively affecting the viability of isolated Langerhans islets. It was shown that islets that partially retain ECM after isolation demonstrate a decrease in apoptosis rate and significantly better support insulin secretion than more aggressively purified islets [4].

Considering the importance of the ECM in ensuring the viability and functioning of Langerhans islets, in the process of decellularization it is important not only to free the matrix from the cellular component, but also, if possible, to keep the structure and composition of ECM as constant as possible. In the future, such a matrix can be recellularized with the corresponding cell types with the prospect of obtaining tissue equivalent of the pancreas with certain functional properties [6].

The pancreas matrix as a total organ was obtained from mice [7, 8], rats [9], pigs [6, 10], and humans [11]. However, the restoration of the vasculature of such intact decellularized organ scaffolds is a complex task. An alternative approach, which is especially relevant for the endocrine transplant of islets, is to combine cells with a decellularized matrix obtained from a pancreatic fragment [3]. Such a strategy may be promising for tissue engineering applications because of its ease of use.

In the present study, we researched the possibility of obtaining tissue-specific pancreatic matrix using small fragments of pancreatic tissue for decellularization. This approach makes it possible to increase the decellularization efficiency, to fully populate with cells the entire volume of the decellularized matrix, to simplify oxygen and nutrients delivery to donor cells, and specifically deep into the matrix, and to significantly reduce the cost of the dellularization procedure.

MATERIALS AND METHODS

Starting material

In the study, the tail part of pancreas obtained as a result of multi-organ retrieval of cadaver organs (n = 6) and unsuitable for transplantation (donor of 34–63 years of age) was used.

Pancreas decellularization

To increase the decellularization effectiveness, a freeze-thaw cycle was introduced into the study protocol to destroy tissue cells at a preliminary stage. Fragments of the donor pancreas were subjected to 1, 2 or 3 cycles of freezing at –80 °C and thawing to +37 °C with the subsequent mechanical grinding of the tissue to 1 × 1 × 1 mm. After that, tissue fragments were processed in three shifts in a buffer solution (pH = 7.4) containing sodium dodecyl sulfate 0.1% solution and an increasing concentration of Triton X100 (1%, 2% and 3%, respectively) (Sigma, USA). In each solution, the sample was kept at room temperature for 24 hours and was constantly stirred at 2 rpm with the CellRoll roller system (INTEGRABiosciencesAG, Switzerland). When changing solutions, the finely dispersed matrix was filtered using a metal sieve (0.4–0.6 mm cell diameter) and returned to the solution with a higher Triton X100 concentration.

At the end of the decellularization process, the pancreas fragments were thoroughly washed off the surfactant residues for 72 hours in phosphate buffer with the addition of an antibiotic / antimycotic. The washed pancreas fragments were out in cryovials, frozen and subjected to γ-sterilization (1.5 Mrad).

Histology

At each stage of decellularization, to control the efficiency of the process, part of the material was fixed in 10% buffered formalin, dehydrated in alcohols of increasing concentration, kept in a mixture of chloroform and ethanol, chloroform and poured into paraffin. 4–5 μm thick sections were obtained with the RM2245 microtome (Leica, Germany) and subsequently stained with hematoxylin and eosin, total collagen (Masson's method) and elastic fibers (Unna–Tenzer method). The cell nuclei were visualized with DAPI fluorescence staining (Sigma, USA). Besides, an immunohistochemical reaction was performed on type I collagen with the anti-collagen I antibody (Abcam, UK) and the Rabbit Specific HRP | DAB (ABC) Detection IHCkit imaging system (Abcam, UK).

Total DNA detection in matrix

The residual amount of DNA serves as an indicator of the cellular components preserved in the decellularized matrix carrying the bulk of the antigens that provide the transplant rejection reaction [12].

Before the study, the samples were stored at –20 °C. DNA was isolated with the DNeasy Blood & Tissue Kit (QIAGEN, Germany) according to the manufacturer's instructions.

The quantification of double-stranded DNA was performed with the Picogreen Quant-iT fluorescent dye™ (Invitrogen, USA) in accordance with the manufacturer's instructions. Briefly, 50 μl of the lysate of the test sample was diluted 1:1 with TE buffer solution, and then

added to 100 μl of the dye solution. For 5 minutes, the resulting solution was incubated at room temperature without access of light, then the samples were activated by radiation with 480 nm wavelength and then analyzed with the Spark 10M microplate reader (Tecan Trading AG, Switzerland) at 520 nm wavelength. To determine the absolute DNA amount, the bacteriophage λ DNA (Invitrogen, USA) calibration curve was used (0 ng/ml – 1000 ng/ml).

Matrix cytotoxicity study

The cytotoxicity of matrix samples in the form of fragments of decellularized human pancreatic tissue *in vitro* was evaluated by direct contact according to the GOST ISO 10993-5-2011 international standard on the mouse fibroblast culture line L929 [13]. The culture medium with 10% fetal calf serum (ETS, HyClone, SV30160.03, USA) served as the negative control. The positive control sample was a single-element aqueous standard of 10,000 μg/ml (Sigma-Aldrich, USA). All procedures were performed under aseptic conditions. Culture monitoring was performed with the Eclipse TS100 (Nikon, Japan) inverted microscope.

The metabolic activity of fibroblasts after contact with matrix samples was determined after 24 hours with the prestoBlue™ CellViabilityReagent (Invitrogen™, USA) according to the protocol recommended by the manufacturer. Changes in media absorption were recorded with the Spark 10M microplate reader with SparkControl™ Magellan V1.2.20 software at 570 nm and 600 nm.

The data quantitative and statistical processing was performed with MicrosoftExcel 2007. All results are presented as mean ± standard deviation. Differences were considered significant at $p < 0.05$.

Study of the functional properties of the matrix

The functional properties of the decellularized human pancreas (DHP) matrix with respect to its ability to maintain cell adhesion, proliferation, and differentiation were studied in a culture of human fat tissue mesenchymal stromal cells (HFTMSC). HFTMSCs were cultured (2^{nd} passage) at 15 × 104 cells / 10 mg of the matrix. After 6 days of cultivation, several samples were taken to assess the HFTMSC viability with the LIVE/DEAD® Cell Viability / Cytotoxicity Kit (Molecular probes® by Life technologies™, USA). After 15 days of cultivation, the remaining matrix samples with cells were fixed in 10% buffered formalin for further histological analysis.

RESULTS

Histology of native pancreas

A morphological study of native pancreas showed the three studied glands bearing signs of expressed lipomatosis, while signs of diffuse fibrosis of pancreatic tissue were found in three other glands (Fig. 1).

Histological analysis of tissue with lipomatosis at different stages of pancreas fragment decellularization

We studied 3 series of samples of native pancreas with signs of lipomatosis, which differ in the number of freezing and thawing cycles. Histology showed that at the initial stage of treatment, after single freezing, in pancreas tissue with lipomatosis, partial damage to the pancreatic parenchyma cells was observed, expressed in the destruction, primarily, of acinar tissue cells. At this, the borders between the cells directly in the acini looked blurred, blurred, and the cell nuclei were not visualized or were pycnotic. However, the contours of the acini were still clearly defined. The Langerhans islets, on the whole, looked structurally more intact, although a significant part of the islet cells nuclei was hyperchromic. It should be noted that at this stage, the process of cell destruction was not global in nature and involved no more than 50% of the studied fragment of pancreatic tissue (Fig. 2, a, b).

The fragments of pancreas with lipomatosis which underwent two freezing / thawing cycles significantly differed in morphological features from the previous series of samples. First of all, this concerned the total spread of the process of cell destruction to the entire tissue fragment. Not even a partial preservation of morphological signs of the initial structure of pancreatic tissue was observed: acini, islets, their fragments were not detected. In the samples, only single preserved cells scattered in the thickness or individual pyknotic nuclei were determined. At this, the stroma was abundantly masked by small grains of cellular detritus (Fig. 2, c, d). It was decided to use the samples obtained in this way for further processing with surface-active agents in order to obtain purified ECM pancreas. As a result of the decellularization, the connective tissue framework was able to get rid of the bulk of the detritus and to obtain samples characterized by a fine-fiber openwork structure, where, however, the minimum inclusion of cell detritus grains was locally revealed (Fig. 3, a, b).

In the samples of pancreas fragments with lipomatosis, after three consecutive freezing-thawing cycles, unlike previously studied samples, preserved cells, cell nuclei, and karyorhexis products were not found. At this, the stroma was disguised by finely divided detritus fragments (Fig. 2, d, e).

At the subsequent decellularization of tissue with lipomatosis, after three freezing-thawing cycles, almost complete removal of detritus grains was observed (Fig. 3, d) and, as a result, a purified fine-fiber matrix was obtai-

ned where blue collagen fibers were distinctly detected by Masson staining (Fig. 3, b). The performed immunohistochemical staining also confirmed the presence of type I collagen in the ECM, which is the most important component of the pancreatic tissue matrix (Fig. 3, a). At orcein staining, red-brown elastic fibers were noted (Fig. 4, a, b). These results indicate the preservation of the main fibrillar proteins of the pancreatic tissue matrix.

Histology of fibrous tissue at different stages of pancreatic fragment decellularization

Already at the first stages of treatment of the pancreas with fibrosis, a significant difference was revealed in the histological pattern from pancreas with lipomatosis, even after three freezing / thawing cycles, a significant number of cells with pycnotic nuclei remained in the tissue fragments (Fig. 2, g, h).

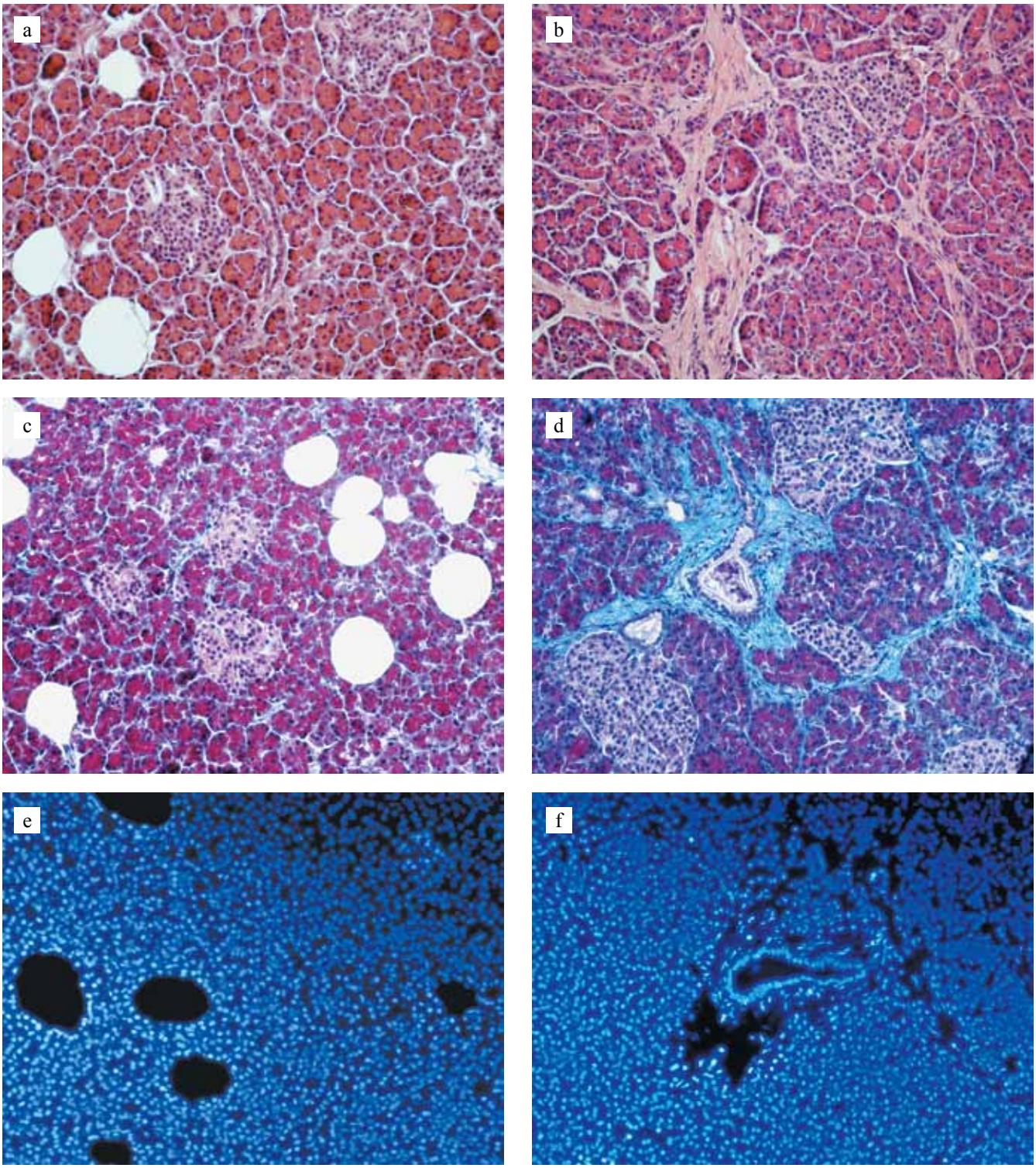

Fig. 1. The histological pattern of human pancreas: a, b, e – native donor pancreas with lipomatosis features; b, d, f – native donor pancreas with fibrosis features; a, b – hematoxylin and eosin; c, d – Masson's method; e, f – nuclear DAPI staining. ×200

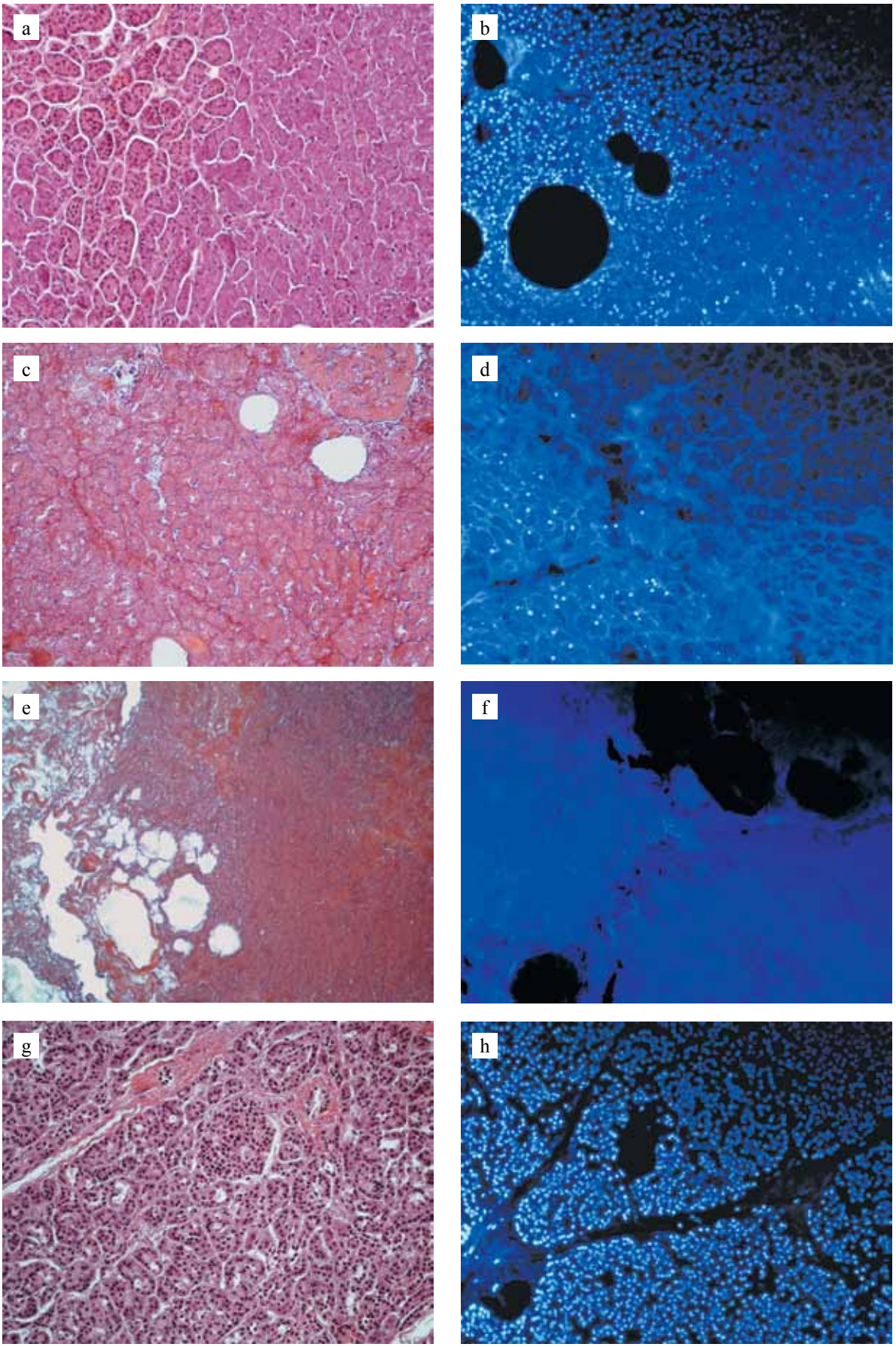

Fig. 2. The histological presentation of pancreatic tissue after successive cycles of freezing and thawing: a, b – 1 cycle of freezing up to –80 °C and thawing up to +37 °C; c, d – 2 cycles; e, f – 3 cycles of freezing and thawing of pancreas with lipomatosis; g, h – 3 cycles of freezing and thawing of pancreas with fibrosis; a, b, e, f – hematoxylin and eosin; b, d, e, h – nuclear DAPI staining. ×200

Nevertheless, such samples were further processed with surface-active agents to confirm the assumption that the developed protocol of decellularization is unsuitable for pancreas with diffuse fibrosis. Indeed, in the obtained samples there were areas with a large number of preserved cells and nuclei (Fig. 3, e, f), while with a similar treatment of the pancreas with lipomatosis, it is possible to obtain a well-purified fine-fiber matrix.

Total DNA quantification in DP matrix

Quantitative analysis showed that in case of pancreatic decellularization with diffuse fibrosis, according to

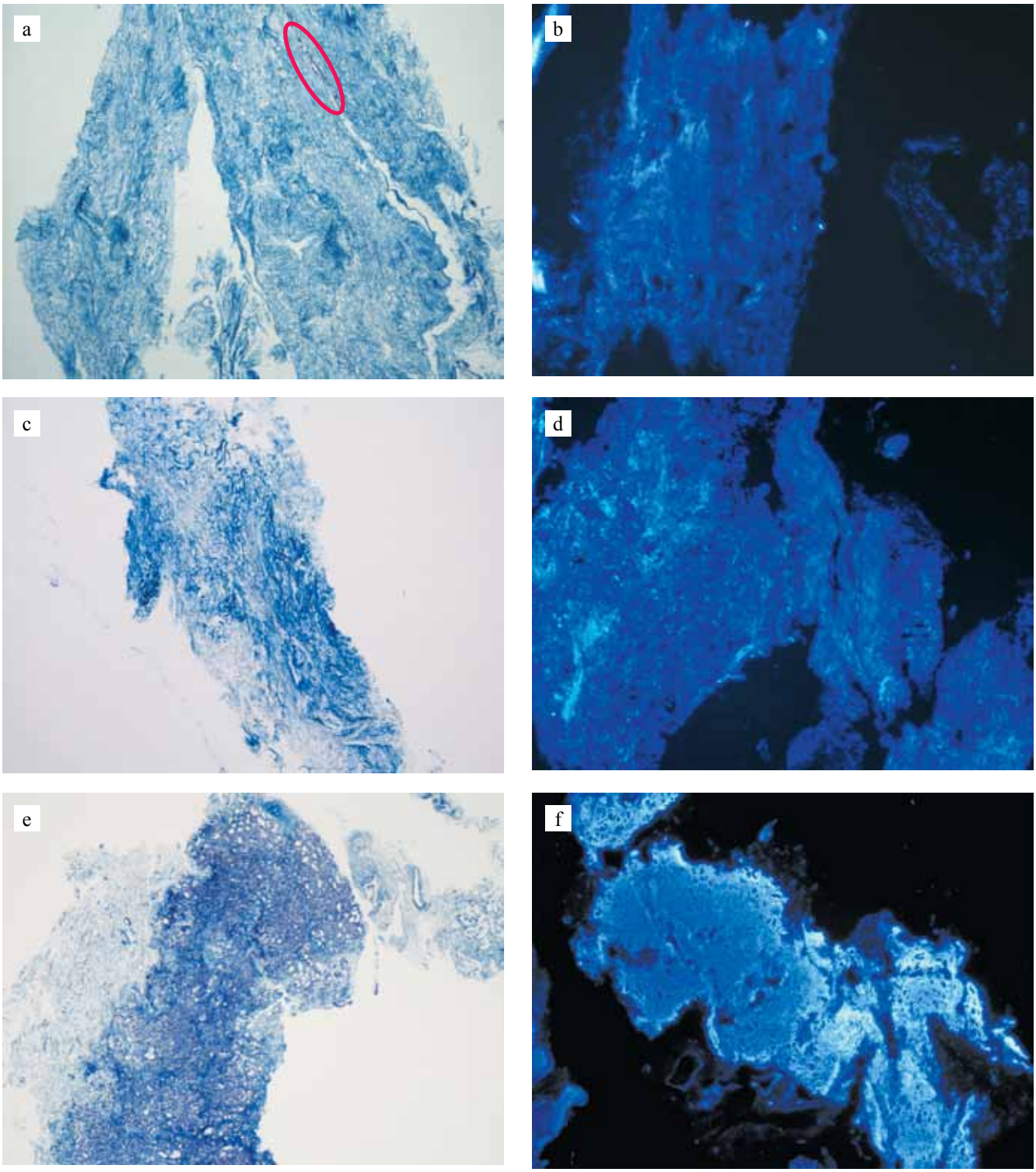

Fig. 3. The histological presentation of decellularized pancreas (DP): a, b – DP with lipomatosis after 2 cycles of freezing and thawing, the red oval marks the area with microfragments of cellular detritus; c, d – DP with lipomatosis after 3 cycles of freezing and thawing; e, f – DP with fibrosis after 3 cycles of freezing and thawing; a, c, e – Masson method; b, d, f – nuclear DAPI staining. ×100

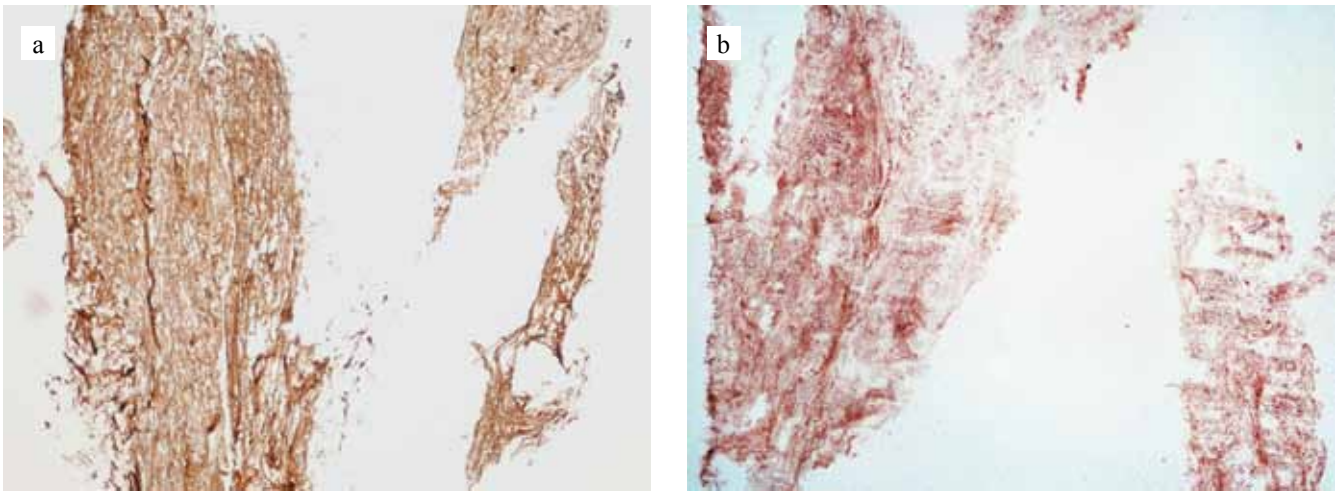

Fig. 4. The histological presentation of decellularized pancreas with lipomatosis: a – immunohistochemical staining demonstrates the presence of type I collagen in the matrix; b – Unna–Tenzer staining reveals the presence of elastic fibers in the matrix. ×100

the developed protocol, 6128.3 ± 718.0 ng DNA/mg of tissue (41.5% DNA) were preserved in the tissue. In this, after the pancreatic pancreatic gland with lipomatosis, we managed to clear the tissue off DNA to a significant extent ($p < 0.05$): the DNA content decreased from 14782.2 ± 319.9 ng/mg of tissue to 12.6 ± 0.9 ng/mg of tissue (Fig. 5), which is 0.1% DNA indicating the high efficiency of the developed protocol of decellularization and low immunogenicity of the resulting matrix, respectively.

Based on the results obtained, for further studies, we used the DP matrix obtained according to the proposed decellularization protocol only for pancreas fragments with detected signs of lipomatosis.

Cytotoxicity of DP matrix

The results were analyzed according to an evaluation scale of the degree of response of cells after incubation with matrix samples in accordance with GOST ISO 10993-5-2011 (Table 1). The negative control corresponds to reaction degree 0, the positive control – to 3 or 4. The degree of response of the test sample should not exceed 2.

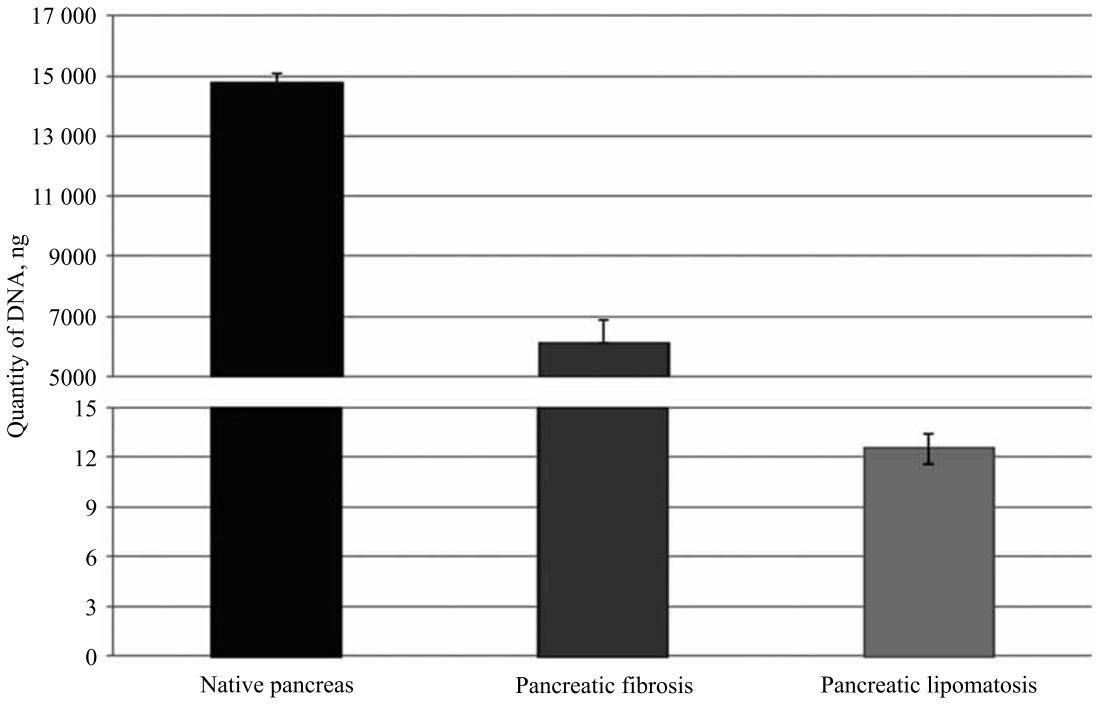

Fig. 5. Quantification of DNA content in native tissue, in the pancreas with fibrosis and with lipomatosis after decellularization

Table 1

Degrees of cell response

Degree	Response	Observed	
0	Absent	Single intracytoplasmic granules Over 90% of proliferating cells	No lysis
1	Insignificant	Not more than 20% of the cells are round, loosely attached, without intracytoplasmic granules Over 80% but under 90% of proliferating cells	Lysis not over 20%
2	Indistinct	Not more than 50% of the cells are round, without intracytoplasmic granules Over 50% but under 80% of proliferating cells	Lysis not over 50%
3	Moderate	Not more than 70% of the monolayer contains round cells Over 30% but under 50% of proliferating cells	Lysis not over 70%
4	Distinct	Almost completely destroyed monolayer Under 30% of proliferating cells	Lysis over 70%

Table 2

Percentage of viable fibroblasts of L929 line relative to the negative control

Sample No.	Sample name	% viable cells relative to the negative control ± sigma	Degree of cell response
1	DP, donor 1	97.3 ± 8.9	0
2	DP, donor 2	90.5 ± 3.9	0
3	Positive control	7.8 ± 2.3	4

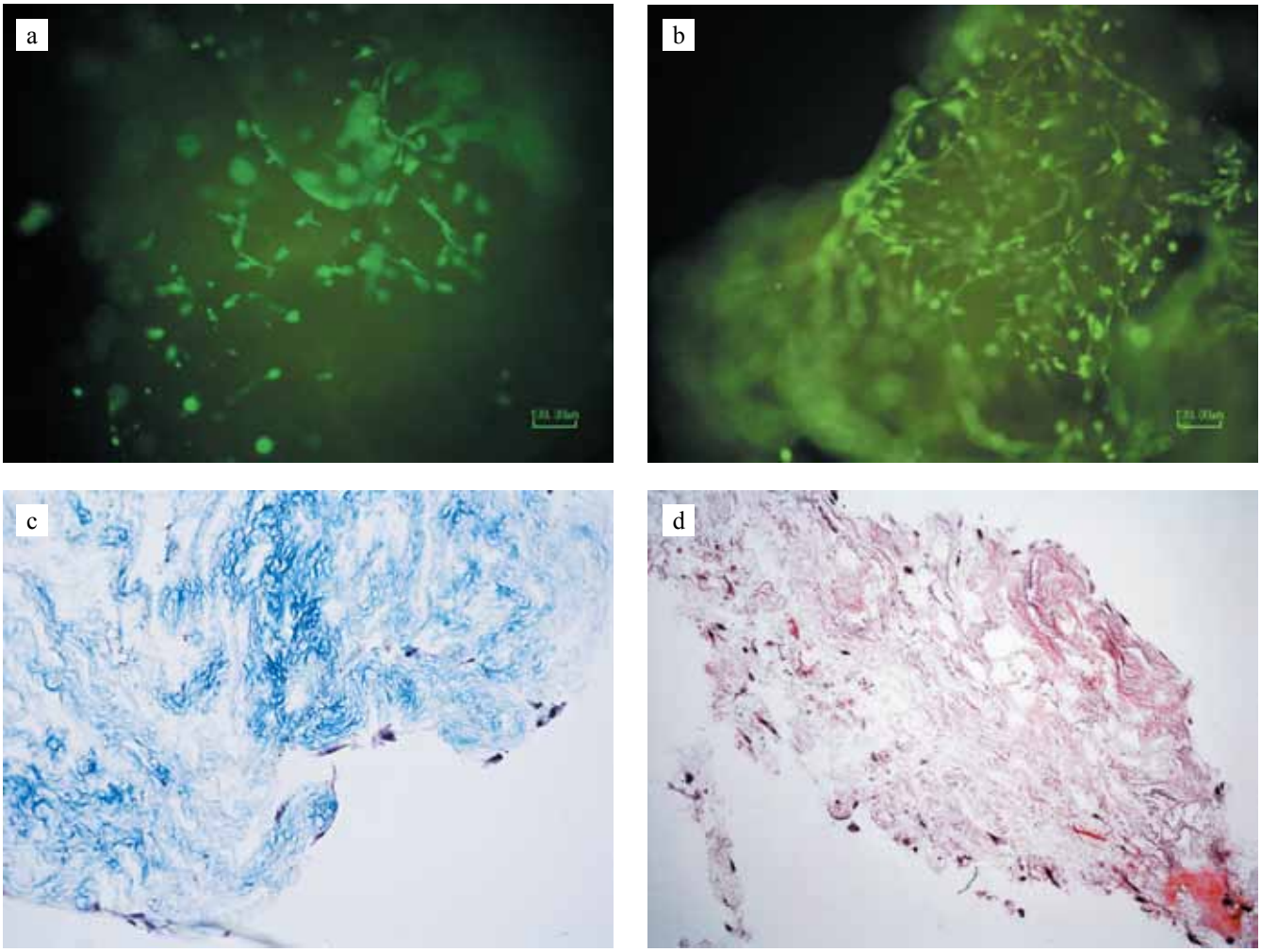

Fig. 6. Culturing of hADMSCs on tissue-specific pancreas scaffold: a – intravital staining of hADMSCs with LIVE/DEAD® vital stain after 1 day of culturing; b – intravital staining of cells with LIVE/DEAD® after 6 days of culturing; c – hADMSCs after 15 days of culturing on DP-matrix, the Masson method. ×200; d – hADMSCs after 15 days of culturing on DP-matrix, hematoxylin and eosin. ×100

Table 2 shows the values characterizing the viability of L929 fibroblasts relative to the negative control, the culture medium with 10% ETS. After contacting, the fibroblast viability remains above 90% which corresponds to the response degree of 0 and indicates the absence of the cytotoxic effect of the samples of the studied matrices. The positive control showed sharp cytotoxicity with the response degree of 4.

Functional properties of DP matrix

Already on the first day of cultivation (Fig. 6, a), HFTMSC on the DP matrices showed cell adhesion and spreading. By the 6th day, the HFTMSC number increased, there were no non-viable cells with their nuclei stained red (Fig. 6, b).

Histology of the samples showed that after 15 days of cultivation, the HFTMSC matrix was intensively populated. The cells are flattened, have a fibroblast-like shape characteristic of this type of cells, are not located only on the surface, but also actively penetrate into the deep layers of the matrix (Fig. 6, c, d).

CONCLUSION

Thus, the criterion for the selection of source material for the effective decellularization of human pancreas is defined. The advantage of using pancreas with lipomatosis was revealed compared with fibrosed pancreas. The proposed protocol for the decellularization of pancreatic donor fragments with lipomatosis is effective and allows one to obtain a tissue-specific matrix / framework free of cells and cell fragments, with a low DNA content and preservation of the morphofunctional properties of ECM pancreas. The resulting matrix does not show signs of cytotoxicity, supports HFTMSC adhesion and proliferation, and can be further used for iscellularization by islet cells (insulocytes, endocrine cells of Langerhans islets) when creating the bioengineered pancreas construct.

The authors declare no conflict of interest.

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INTRASPLENIC IMPLANTATION OF TISSUE-ENGINEERED PANCREATIC CONSTRUCT IN EXPERIMENTAL DIABETIC RATS

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Objective: to study the effect of intrasplenic implantation of a tissue-engineered pancreatic construct (TEPC) on experimental diabetes mellitus. **Materials and methods.** Floating islet-like cultures (FICs) were obtained from the pancreas of newborn rabbits. To form TEPC, FICs were incubated with biopolymer microheterogeneous collagen-containing hydrogel (BMCH). TEPC samples were injected into the splenic pulp of rats with streptozotocin-induced diabetes. **Results.** TEPC with insulin-producing activity was formed on the 7–10th day of incubation of FICs with BMCH. After TEPC implantation in recipient rats, persistent decrease in hyperglycemia and disappearance of clinical signs of diabetes were noted. Histological analysis revealed the presence of groups of islet cells without signs of immune cell response at the TEPC implantation site. **Conclusion.** Our findings indicate that xenogeneic islet cells that were part of the TEPC of the pancreas can survive and actively function after implantation in the splenic pulp of diabetic rat.

Keywords: pancreas, newborn rabbits, floating islet-like cultures, biopolymer microheterogenous collagen-containing hydrogel, tissue-engineered construct, rats, streptozotocin-induced diabetes, splenic implantation, glycemia.

INTRODUCTION

According to the International Diabetes Federation (IDF), 415 million people worldwide have diabetes. This number is predicted to rise to 642 million by 2040 (http://www.diabetesatlas.org). Sources estimate that by the year 2050, as many as one in three adults in the US could have diabetes. The treatment of patients with type 1 diabetes (T1D), who constitute about 10% of all people with diabetes, has fundamentally changed over the past century – daily insulin injection remains the only way to save life. Moreover, the use of intensive insulin therapy with various synthesized hormone drugs cannot protect against development and steady progress of late diabetic complications, such as retinopathy, nephropathy and neuropathy, which are the main causes of disability and premature death in diabetic patients [1, 2]. A natural direction in medicine is the development of methods capable of compensating for β-cells absent in T1D (due to autoimmune destruction) in the pancreatic islets by administering donor islet cells, whose active function, in addition to reducing the need for insulin drugs (up to complete withdrawal for a certain period of time), can lead to regression of diabetic angiopathies. However, islet allotransplantation, which is considered the most effective substitution treatment method, can hardly be called a promising option because of the insurmountable shortage of islet sources – pancreas donated after death. Moreover, in order to attain insulin independence, it is usually necessary to use from two to four donor organs [3], since a significant number of islets are damaged during multi-stage isolation from exocrine pancreatic tissue, as well as in the first 3 days after infusion into the portal vein, during which up to 60% of the transplanted islets are lost. The causes of such losses are, first of all, inflammatory reaction at implantation sites and lack of adequate vascularization. In addition, intraportal administration remains an unsafe infusion method and requires limiting the injected cellular material mass due to the risk of massive portal vein embolization. In this regard, it is expedient to search for safer ways of introducing islets (islet cells), provided there is a favorable microenvironment and sufficient blood supply to the implant. In this connection, there are some hope on creation of a tissue-engineered pancreatic construct (TEPC), which can solve the above problems to some extent.

Earlier, we published some materials on TEPC experimental model [4] and the outcomes of its intraperitoneal implantation. In the present study, we have investigated the effect of intrasplenic implantation of TEPC on experimental diabetes mellitus in rats. Observation of animal recipients was limited to 4 weeks, since persistence of transplant functioning signs during this period allows us to consider its persistent engraftment in the recipient's body to be a fait accompli. The choice of this administration option was primarily due to the possibility of de-

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termining the fate of the introduced TEPC by histologic examination of the implantation site (spleen pulp).

MATERIALS AND METHODS

Obtaining tissue-engineered pancreatic construct

Donor animals (1–3-day-old newborn rabbits) were brought from a specialized nursery owned by the Research Center for Biomedical Technologies, Federal Medical and Biological Agency. TEPC was prepared by joint incubation of floating islet-like cultures (FICs) obtained from the pancreas of newborn rabbits using the method developed by us [4] and through biopolymer microheterogeneous collagen-containing hydrogel (BMCH). Home-made *Sfero*®GEL [5] was used as BMCH.

Formation of tissue-engineered construct was monitored using a Nikon Eclipse TS100 inverted microscope by almost daily monitoring. Significant changes were recorded using a digital camera.

A culture scraper was used to collect TEPC immediately prior to implantation.

Preparation of animals with experimental diabetes mellitus

Male Wistar rats weighing 200–240 g body weight were brought from a laboratory animal nursery owned by Manikhino Experimental Production Facility. Experimental T1D was induced by fractional administration of streptozotocin (70 mg/kg – 12 mg/kg for 5 consecutive days), which, according to our data [6], ensures a stable diabetic status. All manipulations with animals were carried out according to the rules adopted by the European Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes (ETS 123) Strasbourg, 1986. Postprandial glycemia in rat capillary blood was determined using a One Touch Ultra glucometer (Life Scan Johnson & Johnson, USA).

Of 16 rats with streptozotocin-induced diabetes, two groups were formed: Group 1 (control) – 8 rats that did not undergo any treatment, and Group 2 (experimental) – 8 rats that underwent TEPC intrasplenic implantation.

TEPC implantation technique

Each of the Group 2 rats was injected with a TEPC sample. The tissue component contained FICs obtained from 10 pancreas of newborn rabbits.

A TEPC sample was injected into the experimental anesthetized animals (Zoletil, intraperitoneally at a dose of 20 mg per 1 kg of body weight) as follows. After midline laparotomy, the spleen was gently removed into the surgical wound area and placed on a sterile gauze napkin. Using TEPC silicone catheter in the form of a cellular gel composition suspended in 1.0–1.5 ml of medium 199, it was taken ex tempore into a 2 mL syringe. After disconnecting the catheter, an injection needle with a diameter of at least 1 mm was put in its place. The needle was gently pierced into the spleen surface, and a TEPC suspension was slowly injected into the pulp of the organ. To stop bleeding and prevent release of introduced cells, the injection site was tightly closed with a sterile gauze swab for 2–3 minutes. The spleen with the implant was gently returned to the peritoneal cavity. Then the abdominal wall was sutured in layers and the surgical wound was treated with iodine solution.

Histological examination

Control examination of paraffin slices of FICs samples was performed by haematoxylin and eosin staining, as well as by immunohistochemical and immunofluorescence staining with insulin antibodies.

Excised spleen fragments of recipient rats, presumably corresponding to the TEPC injection site, were fixed in formalin. After appropriate treatment of this material and preparation of paraffin blocks, the slices were prepared, which were stained with classic dyes (hematoxylin and eosin), as well as with Mallory's trichrome stain.

RESULTS AND DISCUSSION

TEPC formation

Data from morphological examination of FICs as a tissue component of TEPC showed good morphological integrity with the presence of hormone-secreting beta cells (Fig. 1–3).

After 8–10 days of formation of FICs, they were incubated with biomatrix (BMCH). During co-incubation, the FICs settled to the bottom of a culture vial evenly coated with the biomatrix. Contact with the latter had a beneficial effect on the cultures. They were successfully attached to the BMCH (Fig. 4) with subsequent formation of single-layer growth zones around the FICs

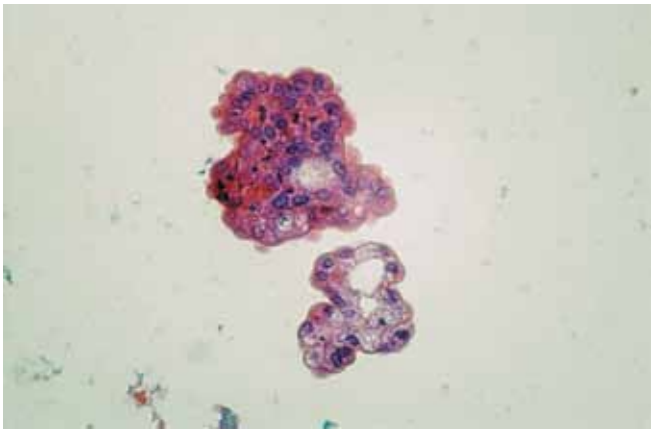

Fig. 1. Floating islet-like cultures, obtained from the pancreas of newborn rabbits, 10th day of incubation. Hematoxylin and eosin staining. ×200

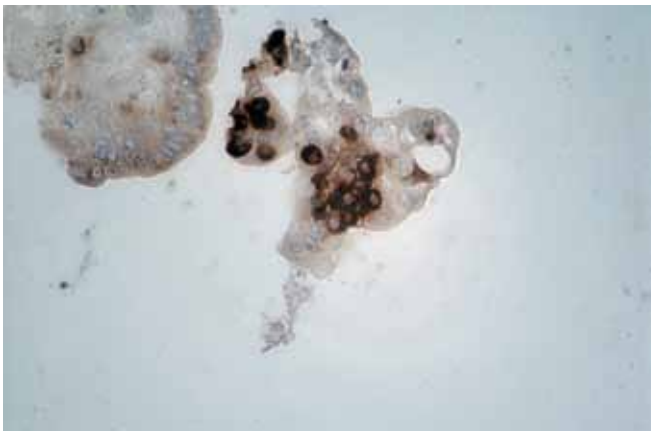

Fig. 2. Floating islet-like cultures, obtained from the pancreas of newborn rabbits, 10th day of incubation. Immunohistochemical staining with insulin antibodies. ×200

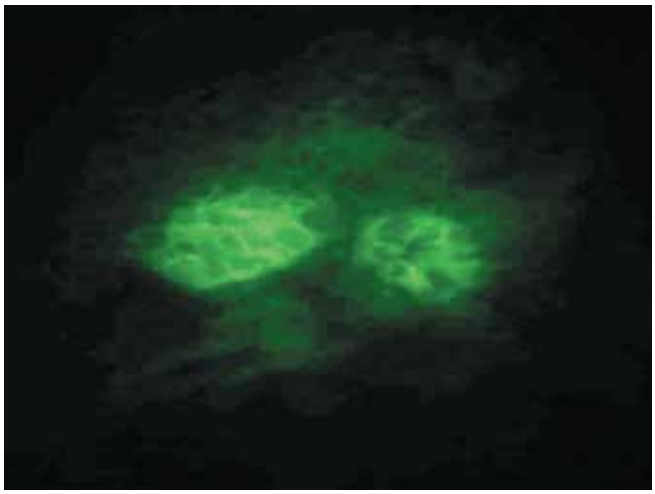

Fig. 3. Glow of insulin granules in floating islet-like pancreatic cultures. Immunofluorescence staining with insulin antibodies. ×200

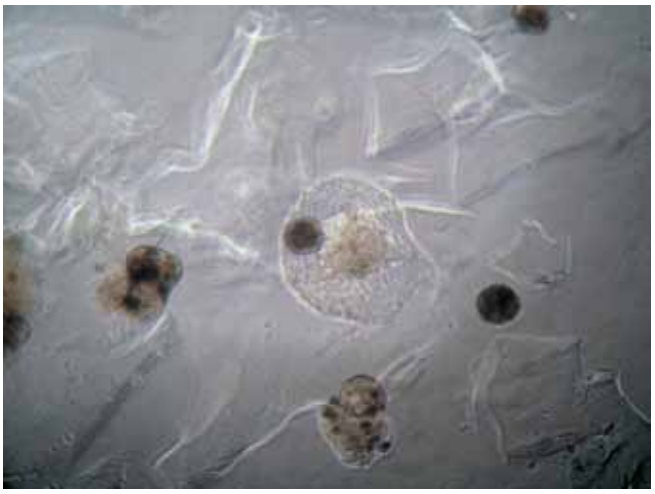

Fig. 4. Attachment of flotation islet-like cultures to matrix. The inverted microscope. ×40

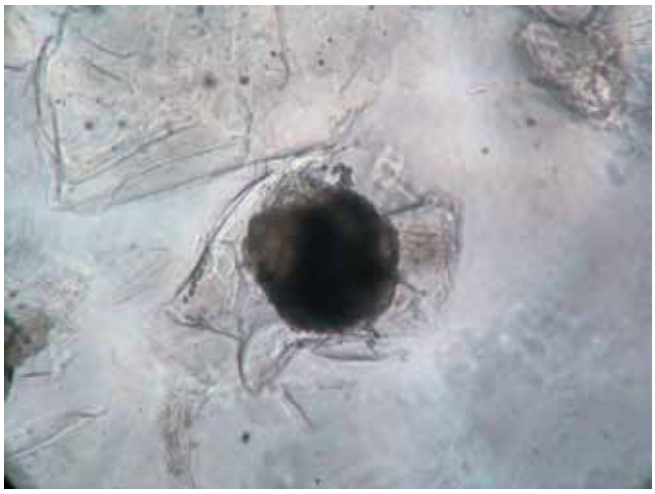

Fig. 5. Formation of pancreatic tissue engineering construct. The inverted microscope. ×100

adhesion site, which indicated good matrix properties of BMCH relative to FICs. Co-cultivation of FICs and biomatrix *in vitro* resulted in TEPC formation (Fig. 5).

Animal condition and blood sugar test results

In group 1 (control diabetes), 2 weeks after streptozotocin administration, all 8 animals showed characteristic clinical signs of diabetes: weight loss, polydipsia, polyuria, lethargy, hair loss and hair yellowing. Such pronounced diabetic status corresponded to high blood sugar levels – from 22.5 to 32.4 mmol/L (Table 1). None of the rats in this group showed a tendency to significantly reduced hyperglycemia, which testified to the reliability of the T1D model used in this study. At the same time, two rats (#17 and #24) with the highest levels of hyperglycemia during the observation died on the background of extreme exhaustion.

In Group 2 (experimental), the pre-transplan blood sugar level 2 weeks after the last streptozotocin injection was almost the same as in the control diabetic animals at the same time (Table 2). However, a week after TEPC implantation, there was a clear tendency toward decreased hyperglycemia. A week later, the average blood sugar level in the recipient rats was significantly reduced ($p < 0.05$), dropping to 17.1 mmol/L. During this period, three animals with relatively moderate reduction in hyperglycemia (#13, #19 and #21) were euthanized to histologically examine the spleen fragment, where the TEPC sample was supposedly injected. The remaining recipient rats were followed up for 4 weeks after implantation. Although none of the experimental rats had normalized blood glucose concentrations, glycemia did not return to its high pre-transplant levels. Moreover, in all Group 2 animals, the pronounced clinical signs of diabetes practically disappeared, a slow but steady in-

Table 1

Changes in blood sugar levels (mmol/L) in control group rats (#1) (streptozotocin-induced diabetes without treatment)

S/n of rat	Weeks before (–) and after (+) implantation in Group 2						
	–2	–1	**0**	+1	+2	+3	+4
2	25.1	23.3	**22.0**	24.5	23.9	22.5	25.4
5	26.2	24.4	**26.3**	25.1	24.8	25.0	25.4
6	22.5	23.7	**24.1**	23.2	23.4	22.8	23.6
9	26.9	27.4	**26.0**	28.5	27.9	27.5	26.4
17	32.4	>33.3	**>33.3**	death			
23	27.7	30.0	**28.7**	27.3	28.1	25.5	26.4
24	31.8	32.5	**›33.3**	>33.3	>33.3	death	
29	28.1	28.5	**26.1**	27.2	27.9	27.5	28.4
M	**27.6**	**27.1**	**25.9**	**25.8**	**25.4**	**25.5**	**26.2**

Table 2

Changes in blood sugar levels (mmol/L) in Group 2 rats after implantation of tissue-engineered pancreatic construct into spleen pulp

S/n of rat	Weeks before (–) and after (+) implantation						
	–2	–1	**0**	+1	+2	+3	+4
3	25.2	23.9	**24.3**	19.8	16.4	17.7	14.0
4	29.6	25.8	**25.7**	19.2	15.3	13.9	14. 1
11	20.3	21.6	**22.0**	19.9	12.8	14.9	12.5
13	26.9	23.2	**26.5**	24.9	18.4	euth.	
19	27.7	23.1	**26.0**	21.4	16.1	euth.	
20	24.4	28.6	**31.1**	26.6	14.7	15.8	16.4
21	25.3	24.7	**27.2**	22.1	21.9	euth.	
28	33.1	32.2	**31.1**	28.2	21.1	21.4	21.0
M	**26.5**	**24.9**	**25.5**	**22.8**	**17.1**	**16.7**	**15.6**

crease in body weight began, including in rat #28, which had the highest glycemia level before transplantation (31.1 mmol/L) Apparently, a 1/3 decrease in blood glucose concentration allowed the animal to adapt to daily relatively high glycemia and maintain viability. Clinical remission of the diabetic status in Group 2 animals remained until the end of post-implantation observation.

Data from histological examination of the spleen of recipient rats

Results from morphological examination of the spleen of recipient rats were of particular interest, as it was possible to determine to some extent the fate of the TEPC implanted in rats with experimental T1D. At the same time, 3 rats from Group the 2 were first euthanized for subsequent histological examination of the spleen. This was to first identify the post-implantation state of TEPC in the short-term period after administration (2 weeks). In one of these rats (#21), in which hyperglycemia decreased slightly (see Table 2), implant-like structures were not detected, but signs of spleen pulp trauma in the form of spleen pulp ruptures were revealed. Apparently, during administration of the TEPC-containing suspension through the ruptures made by the injection needle, a part of the implant went beyond the spleen and got into adverse conditions. This did not allow the introduced islet cells to manifest themselves sufficiently.

In rat #19 with more pronounced hyperglycemia reduction, structural formations were identified, which can be attributed to fragments of the implanted TEPC (Fig. 6), as they contained both epithelial (islet) cell groups and biomatrix residues surrounded by white blood cell groups, which, apparently, were actively involved in BMCH resorption. In rat #21, which was euthanized also 2 weeks after TEPC administration, spleen pulp sections were found with moderate leukocyte infiltration around a group of islet-like structures and with formation of a soft connective tissue capsule (Fig. 9, 10), which may have been formed during resorption of implanted biomatrix residues.

At the end of the experiment – 4 weeks after TEPC implantation – islet-like implants of epithelial cells were found in two recipient rats (#4 and #11) (Fig. 9, 10). In both animals, glycemia levels by the time of euthanasia decreased (almost twice) when compared to pre-implantation levels, in the absence of characteristic clinical manifestations.

It is important to note that there were no histological signs of cellular immune response to cellular xenograft

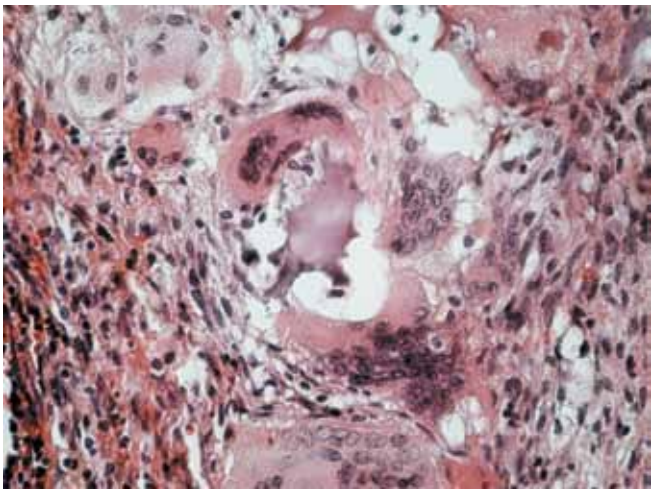

Fig. 6. Spleen from rat #19 two weeks after intrapulpal implantation of TEPC. In the center is a resorbable BMCH fragment with scalloped edges, surrounded by white blood cell groups. ×200

Fig. 7. Spleen from rat #21 two weeks after intrapulpal implantation of TEPC. In the center are groups of islet-like structures and biomatrix residues. Hematoxylin and eosin staining. ×100

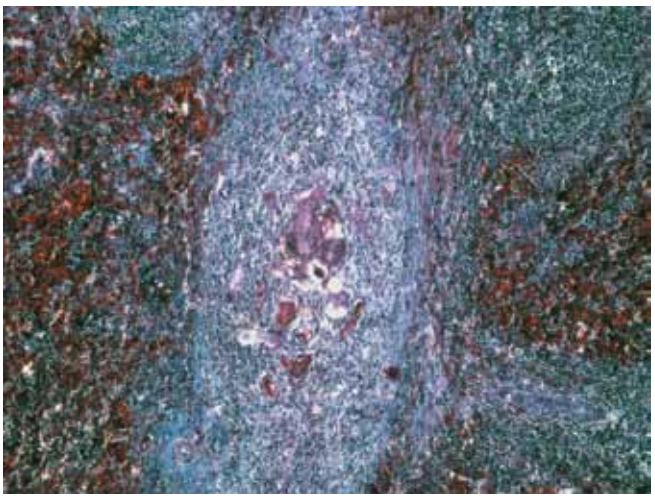

Fig. 8. The same. Mallory's trichrome stain. ×200

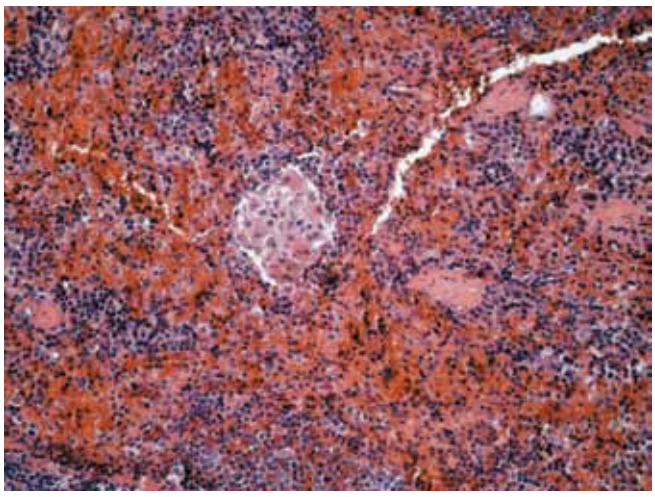

Fig. 9. Spleen from rat #4 four weeks after intrapulpal implantation of TEPC. In the center is an epithelial islet-like structure without signs of destruction and cellular immune response in the absence of BMCH residues. Hematoxylin and eosin staining. ×200

implanted as part of TEPC in the complete absence of biomatrix residues. Apparently, by the end of the 4th week after TEPC implantation in the spleen pulp, BMCH resorption was completely completed and the accompanying leukocyte reaction disappeared. It is possible that the biomatrix that remained for a certain time, to some extent, switched the immune response of the spleen to itself and allowed xenogenic islet-like structures to survive and function for a long time in the body of a foreign recipient. At the same time, islet cell cultures that were part of TEPC themselves, as was shown earlier, have significantly reduced immunogenicity [7].

Thus, experiments on intra-splenic implantation of a TEPC, consisting of islet-like cultures and biodegradable microheterogenous collagen-containing matrix in rats with streptozotocin-induced diabetes, confirmed the morphological integrity and functional activity of TEPC *in vivo*. However, the hypoglycemic effect of TEPC implantation in the spleen was less pronounced compared to intraperitoneal injection of similar TEPC samples, which we had previously performed [4]. Apparently, this difference can be explained by the fact that when a TEPC sample is implanted into the peritoneal cavity, its entire quantity reaches its destination, which is ensured by additional washing of the syringe and injection needle with the aim of introducing the remnants of the cellular gel suspension. However, when spleen is introduced into the pulp, the quantity of TEPC sample introduced is naturally limited, and attempts to increase the implant volume are fraught with organ ruptures and

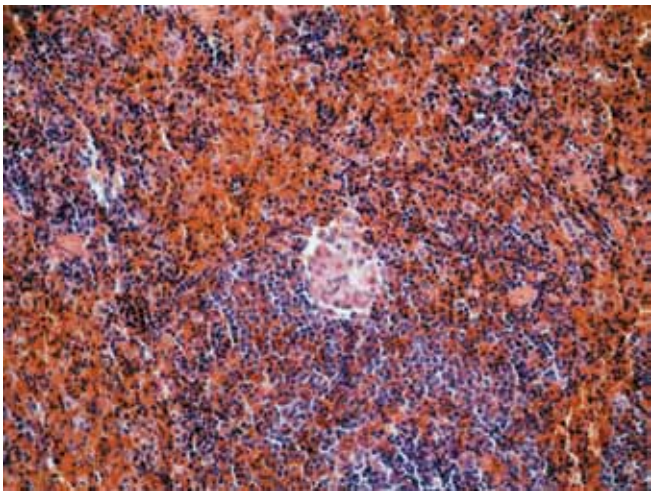

Fig. 10. Spleen from rat #11 four weeks after intrapulpal implantation of TEPC. In the center is an islet-like structure with no signs of destruction and cellular immune response. Hematoxylin and eosin staining. ×200

suspension backflow. Therefore, the mass and functionality of intrasplenic and intraperitoneal implants can vary significantly. At the same time, this study has to some extent achieved its main goal, which could not be achieved with intraperitoneal TEPC administration. If the implant is not found in the peritoneal cavity several weeks before euthanasia, it can be found in the spleen pulp of the rat recipient. Histological analysis conducted showed that two weeks after intrasplenic implantation of TEPC, there was active biomatrix resorption, and two weeks later it was completely dissolved, and the implant was preserved in the form of islet-like structures. Their morphological integrity and absence of signs of cellular immune response made it possible to explain the obtained anti-diabetic effect via the functioning of islet cells that were part of TEPC.

The authors declare no conflict of interest.

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BIOMEDICAL CELL PRODUCT MODEL FOR PRECLINICAL STUDIES CARRIED OUT ON A LARGE LABORATORY ANIMAL

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Objective: to develop a model of a biomedical cell product that is consistent with the "homologous drug" strategy based on protocols for preparing the cell component and scaffold carrier for preclinical studies on a large laboratory animal (pig). **Materials and methods.** Biomedical cell products and skin equivalents (SE), were formed using plasma cryoprecipitate prepared from blood plasma of healthy donors and mesenchymal stem cells (MSCs) of human adipose tissue. Cryoprecipitate from pig blood plasma and human adipose tissue-derived MSCs were used to form model skin equivalents (mSE). Bright-field microscopy, phase-contrast microscopy (Leica DMI 3000B) and fluorescence microscopy (Cytation 5 imager; BioTek, USA) were used to monitor the state of cells in the culture and in the composition of the equivalents. Scaffolds for equivalents were tested for cytotoxicity (MTT test, direct contact method). The cell distribution density was characterized by author's method (Patent No. 2675376 of the Russian Federation). **Results.** An mSE was developed for preclinical studies on a large laboratory animal (pig). In the mSE, components that change from halogen to xenogenic conditions during transplantation to the animal were replaced. A comprehensive approach to preparing mSE was presented. It includes sampling of primary pig biomaterial, extraction and characterization of adipose tissue-derived MSCs, preparation of a scaffold carrier for the corresponding "homologous drug" strategy. Cytotoxicity of the mSE scaffold was evaluated. It was shown that mSE provides mechanical support (similar to SE) to cells, as well as comparable development of cellular events during cultivation. **Conclusion.** A model of a biomedical cell product was developed. This model is consistent with the "homologous drug" strategy for preclinical studies on a large laboratory animal (pig). The paper presented a comprehensive approach to developing a model equivalent based on protocols for preparation and testing of the cellular component, the scaffold carrier and the ready-to-use model equivalent.

Keywords: skin equivalent, scaffold, mesenchymal stem cells, preclinical studies, homologous model.

BACKGROUND

In vivo experimental models used for testing new treatment methods remain the golden standard at the preclinical stages of developing drugs and new products of tissue engineering. Currently in vivo preclinical studies are most commonly performed on small laboratory animals, such as mice, rats and rabbits. At the same time there is no doubt regarding the advantages of using large animal models which result from the fact that they possess organs the size and phenotype of which are comparable to human ones. The latter enables to use equipment and methods which are developed and used in people for implantation, follow-up and analysis of the results of using tissue-engineered products in preclinical studies using animals [1]. Longer periods of life for large laboratory animals enable to perform long-term studies. The results of the studies for animals whose physiological parameters and metabolism rates are similar to those in humans may be extrapolated to people with a sufficient degree of certainty.

While selecting an animal model in order to study biomedical cell products (BMCP) it is necessary to pay special attention to the species-specific features of the animals and to the mission of the BMCP. For example, in the course of studying BMCPs intended for restoration of damaged or missing skin tissues it should be taken into account that there exists an enormous diversity of animal types whose skin structure and anatomy varies greatly. Interspecies anatomic and histological skin differences are extremely significant while selecting an animal model whose skin structure would be close or similar to human skin [2]. Relatively recently pigs have been widely recognized as a model for studying human skin tissue repair, as the anatomic and physiological skin structure of a pig is very similar to that in humans. Unlike free-skin animals (these include rabbits, rats, mice) pigs, same as humans, have a strong connection between the derma and the fascia [3]. The thickness and structure of the epidermis and derma are comparable in pigs and in humans [4]. Another important feature that human and pig skin have in common is the absence of a thick body hair layer. Epidermal enzyme patterns, keratin proteins, histological location of dermal collagen IV, fibronectin and vimentin, turnover time of the epidermal tissue for

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porcine and human skin have a high degree of comparability [5]. Moreover, the orientation and distribution of blood vessels in pig derma are similar to the vessel distribution in human skin [6]. Therefore many specialists believe that pigs are the most relevant animal models to study the effectiveness of using skin equivalents and wound healing.

At the same time while developing the design of preclinical studies not only physiological similarity of the chosen animal model should be taken into account but also the existing differences. For example it is known that pigs' and human immune systems are quite similar. However there is also a number of significant differences [7]. Summerfield A. et al. (2015) have shown that immunocompetent porcine skin cells also possess a set of characteristics which distinguish them from human ones [8]. The regenerative process development in the skin tissues is closely associated with the work of the immune system. The immune response is even more significant when tissue-engineered products are transplanted into the wound defect area. It is generally known that tissue-engineered products are not inert but possess high biological activity. The latter may be related both to the cellular component and to the non-cellular component (artificial extracellular matrix/scaffold). The presence of biological activity inevitably leads to the question regarding histocompatibility and immunogenicity of such products. In the development of tissue-engineered products histocompatibility and immunogenicity are taken into account in reference to the human body. For example often one of the key BMCP components are Mesenchymal Stem Cells (MSCs). They possess a number of properties which stimulate the development of a regenerative process in the damaged tissues and are characterized by hypoimmunogenecity related to absence of class II HLA receptors and MSC immunomodulatory activity [9]. The latter expands the potential of using such constructs under allogenous conditions. However in the course of preclinical studies on animal models tissue-engineered products advance from allogenous to xenogenous conditions, which a priori increases the risk of the recipient's organism with the implanted construct. Therefore in the course of preclinical studies phylogenetical differences should be taken into account even if an animal model which is very close to humans is used. During preclinical studies aimed at proving the effectiveness of BMCP use, in order to minimize negative results due to the body's reaction to the xenogenous component the developed products may be modified towards the 'homologous drug' strategy. In this context developing homologous BMCP models for certain types of laboratory animals is currently important.

Objective of the research: to develop a biomedical cell product model which would be consistent with the 'homologous product' strategy on the basis of protocols for preparing the cellular component and the scaffold carrier for preclinical studies on a large laboratory animal (pig).

MATERIALS AND METHODS

1.1. General principles of the work

All the procedures related to cell isolation and cultivation, working with blood and its derivatives, work aimed at the development and cultivation of hydrogel scaffolds took place at class C premises under sterile laminar flow unit conditions (class A) at the laboratory of biotechnology at the Federal State Budget Education Facility of the Volzhsky Region – the Privolzhsky Research Medical University of the Russian MoH. During the course of cell and hydrogel scaffold cultivation the growth media underwent regular control for sterility, mycoplasmatic and viral contamination, presence of fungal flora. The study protocol was approved by the local ethical committee at the Federal State Budget Education Facility of the Volzhsky Region – the Privolzhsky Research Medical University of the Russian MoH and affirmed by the Academic Council of the same University. Each person included in the study submitted voluntary informed consent.

1.2. Obtaining primary donor material

The peripheral blood of 3 healthy volunteers was used as a source of human blood plasma. The blood was taken in the morning (fasting) from the cubitus vein and placed into 'RAVIMED' polymer containers (Poland) with CPDA-1® hemopreservative solution. The blood plasma was separated by centrifugal method at 3000 rpm during 20 minutes. After centrifugation blood plasma was withdrawn. The obtained blood plasma was frozen and stored at –40 °C. The source material for obtaining mesenchymal stem cells was adipose tissue obtained in the course of cosmetic surgeries at the department of reconstructive and plastic surgery, University clinic of the Federal State Budget Education Facility of the Volzhsky Region – the Privolzhsky Research Medical University of the Russian MoH. The material was obtained from three young women whose mean age was 29.3 years (20 to 34 years old).

1.3. Separation and cultivation of human mesenchymal stem cells

Human mesenchymal stem cells were obtained from human adipose tissue Human Adipose-derived Stem Cells – hASCs) by means of thermal enzyme treatment with collagenase (Sigma-Aldrich, Germany) and cultivated in full growth media (media α – MEM, 20% fetal calf serum (FSC), glutamine, antibiotics penicillin/streptomycin) under absolute humidity, +37 °C, 5% CO_2 (CO_2 temperature regulated chamber, "Sanyo", Japan). The media and agents used were produced by "Gibco®"

(UK), the plastic vessels by "Costar" Co. (USA). After obtaining a subconfluent monolayer (60–70%) the culture was subcultured. Cultures after the 3rd–4th passage were used for experiments. The hASCs used for work had a confirmed differentiation potential in adipogenic, osteogenic and chondrogenic directions. Cell viability before their introduction into the experiment was 98–99%. The immunophenotype of the cells was typical for mesenchymal stem cells: the cells expressed CD 90, CD 105, CD 73, CD 44, CD 10 and did not express CD 45, CD 14, CD 34, HLA DR, which corresponded to the criteria determined by the International Society on Cell Therapy for mesenchymal stem cells.

1.4. Assessment of culture concentration and viability of the cells

Concentration and viability of the cells was counted by means of "Countes" cell counter (Invitrogen, USA), using trypan blue intravital stain (Sigma-Aldrich, Germany).

1.5. Determining the immunophenotype of human mesenchymal stem cells

Before starting the experiment the cell phenotype was determined using the monoclonal antibody panel CD 90 FITC, CD 105 PE, CD 73 PE, CD 44 FITC, CD 45 PC5, CD 34 PC7, CD 14 PC5, CD 10 PC7, HLA-DR PC7 (Becton Dickinson, USA) with appropriate isotypic controls on a BD FACS CANTO II flow cytofluorimeter (Becton Dickinson, USA).

1.6. Obtaining material for porcine mesenchymal stem cells and porcine blood plasma

Mesenchymal stem cells obtained from porcine adipose tissue (Porcine Adipose-derived Stem Cells – pASCs) were used as a test culture. Porcine blood plasma was used in order to determine a model equivalent of skin. The study protocol was approved by the local ethical committee of the Federal State Budget Education Facility of the Volzhsky Region – the Privolzhsky Research Medical University of the Russian MoH (protocol ## 8). All the procedures related to working with animals took place at a vivarium at the operating room, observing aseptic and antiseptic regulations and the requirements stated by the European convention for the protection of vertebrate animals used for experimental and other scientific purposes (№ 123 of 18 March 1986; ETS No. 170, June 22, 1998, Strasbourg)

Three piglets (Landrace breed, age 8 weeks, weight 13–15 kg, female) were used as a source of pASCs. The animals were introduced into the experiment after a 14 day quarantine. Before the operation the animals were carefully washed using a soap solution. At the operating room 10–20 min before general anesthesia premedication was performed using intramuscular administration of XylaVet (Pharmamagist Ltd, Hungary). The material was obtained under combined anesthesia: after intravenous administration of Propofol ("B-Braun", Germany) the animals were intubated, later anesthesia was maintained using Sevorane ("Abbott", UK). During analgesia the narcotic drug Fabius CS ("Dräger Medical GmbH", Germany) was used, pulse and arterial blood oxygen saturation monitoring was performed using a Nihon Kohden monitor (Nihon Kohden, Япония).

The surgical field was successively processed with 5% alcohol iodine solution, then 70% ethyl alcohol. In order to obtain adipose tissue MSCs 10–30 cm^3 of subcutaneous tissue were removed from the animals' dorsal area. Tissues were obtained using an ACCULAN® 3Ti Dermatome GA 670 electrodermatome (Braun, Germany). A layer of skin and subsequently the upper layer of the subcutaneous adipose tissue (1.2 mm) were removed from the surgical field by the electrodermatome. After that subcutaneous adipose tissue samples were obtained (graft thickness 1.2 mm, graft width up to 10 mm, layer depth up to 2.4 mm). The subcutaneous adipose tissue were rinsed repeatedly and carefully in order to remove blood admixtures in Hanks' solution with antibiotics (100 U/ml penicillin, 100 mcg/ml streptomycin, Sigma, Germany).

Blood sampling in order to obtain blood plasma was performed from the femoral vein. Vein catheterization was performed under ultrasound control (SonoSite SLL Ultrasound System, USA). Blood was collected into 'RAVIMED' polymer containers (Poland) with CPDA-1® hemopreservative solution. The procedure of separating porcine blood plasma was similar to the procedure of separating human blood plasma as described in section 1.2. Euthanasia was performed by means of air embolism under anesthesia.

1.7. Separation and cultivation of porcine mesenchymal stem cells

Cells were obtained from adipose tissue by means of mechanical disaggregation and thermal enzyme processing with 0.2% type I collagenase solution (Sigma, USA) during one hour. After that the cells were cultivated in full growth media in 25 cm^2 culture flasks. Media and agents used were produced by "Gibco®" Co. (UK), plastic materials – by "Costar" Co. (USA). The first change of growth media was performed in 72 hours. Later the media were changed twice a week. After obtaining a subconfluent monolayer (60–70%) the culture was subcultured. Cultures after the 3rd–4th passage were used for experiments. Cell cultivation at all stages took place in a CO_2 temperature regulated chamber ("Sanyo", Japan) at 37°C, 5% CO_2, absolute humidity.

1.8. Differentiation potential assessment

Cell differentiation potential was assessed on 3rd passage cultures. The Hyman Mesenchymal Stem Cell Functional Identification Kit (R and D systems, USA) was used for differentiation.

1.9. Determining the immunophenotype of porcine cells

Determining the immunophenotype of porcine cells was performed by multicolour assay method using the direct immunofluorescence method. The monoclonal antibody panel CD 44 FITC, CD 90 PerCP-Cy5.5, CD 10 PC7, CD 45 PE (Becton Dickinson, USA) was used in this work with corresponding isotypic controls on a FACS CANTO II cytofluorimeter (Becton Dickinson, USA). The measurement parameters have been set once and standardized using particles for BD Cytometer Setup and Tracking Beads (BD™CS&T Beads). Stained cells were incubated for 30 minutes and then rinsed in order to later determine the immunophenotype. The results were expressed as the ratio of cells bearing the corresponding marker (in percentage points).

1.10. Forming of BMCP (skin equivalents / hydrogel scaffolds)

In order to form BMCP – skin equivalents (SE) / hydrogel scaffolds (Pat. № 2653434 RF, 11.04.2017; [10]) blood plasma obtained from healthy donors was used. Frozen blood plasma was defrosted at +2 °C and placed into a centrifuge at +4 °C for 15 min at 1500 rpm in order for the cryoprecipitate to settle. Later 85% of the supernatant from the initial frozen blood plasma volume was collected. The cryoprecipitate was placed into a temperature-regulated chamber at 37 °C till full dissolution. The amount of fibrinogen in blood plasma, blood plasma cryoprecipitate and in the supernatant was determined. The cryoprecipitate was standardized by fibrinogen amount till the final concentration 6 g/l was achieved. For standardization purposes supernatant with low fibrinogen content remaining after cryoprecipitate isolation was used. For the forming of skin equivalents / hydrogel scaffolds a blood plasma cryoprecipitate pool from three donors was used.

For pegylation of the protein part of the cryoprecipitate PEG-NHS (Sigma-Aldrich, Germany) was used. A 2% solution of type I acetous collagen was added to the pegylated cryoprecipitate (collagen obtained from cod skins (Pat. № 2567171 RF… dated 06.10.2014) [11]) which had been pre-neutralized with NaOH. To the obtained composite a gentamycin solution was added – 0.26 mg/ml of the composite (Therma Fisher, USA). Later a cell (hASCs) suspended mixture in a phosphate-buffer saline (PBS) was added to the composite at the ratio of 7:1 correspondingly. The cell concentration was 1.2×10^5 per 1 ml of the composite. The resulting composite was transferred to a form – a 3.5 cm diameter Petri dish pre-treated with silicon. In order to achieve polymerization of the composite a thrombin-calcium mixture was added to it: 80 ME/ml of bovine thrombin (Sigma-Aldrich, Germany) in a 1% $CaCL_2$ solution.

The resulting skin equivalents (SE) were kept in a form for 20 min at +22–25 °C. Then they were transferred to cultural dishes (Corning 60 mm × 15 mm; USA) and covered with 6 ml of full growth media. The cultural dishes with the SE were transferred to a CO_2 temperature regulated chamber (37 °C, with humidified atmosphere and 5% CO_2 content) and cultivated during 6 days, the growth media being changed twice a week [12].

In order to form a model skin equivalent (mSE) as part of SE during its formation the human blood plasma cryoprecipitate and hASCs were replaced with porcine blood plasma cryoprecipitate and pASCs. The method for obtaining porcine blood plasma cryoprecipitate (a pool from three animals) and the method of forming mSE were identical to methods for obtaining human blood plasma cryoprecipitate and forming SE.

Studies ## 1.12.1 and 1.12.2 were carried out on acellular hydrogel scaffolds formed on the basis of human blood plasma cryoprecipitate and or on the basis of porcine blood plasma cryoprecipitate. Scaffolds were formed according to the method described above and under the same conditions. In the course of forming acellular scaffolds cell suspended mixtures in the PBS were substituted by an equal volume of PBS. Acellular hydrogel scaffold samples were used in the studies immediately after production.

1.11. Microscopy

The human and porcine cell structure condition was followed up at all cultivation stages. In order to observe the condition in the hydrogel scaffold structure a bright field and phase contrast method were used. Microscopy and video archiving were performed by means of Leica DMI 3000B inverted microscope equipped with a LAZ.V.4.3 image visualization programme.

In order to visualize the cells and confirm their viability in the scaffold structure fluorescent microscopy performed on a Cytation 5 image tool (BioTek, USA). In order to visualize viable cells Calcein AM was used (catalog № 564061, BD). In order to visualize cell nuclei (cytoblasts) Hoechst 3334 (USA) was used. Staining was performed in accordance with the manufacturer's protocol.

1.12. Studying the impact of hydrogel scaffolds on the hASCs culture

1.12.1. Cytotoxicity assessment – MTT test

The samples studied were cell-less hydrogel scaffolds (6 scaffold samples on the basis of human blood plasma

cryoprecipitate – SE scaffold and 6 scaffold samples on the basis of porcine blood plasma cryoprecipitate – mSE; the sample diameter was 33 mm, thickness – 2 mm). Each sample was placed into cultural dishes (Corning 60 mm × 15 mm; USA) and covered with 6 ml of full growth media. The samples in the media were placed into a CO_2 temperature regulated chamber and incubated under standard conditions for 1 day (3 SE scaffolds, 3 mSE scaffolds) and for 8 days (3 SE scaffolds, 3 mSE scaffolds) in order to obtain an extract. After the control period (1 day, 8 days) the extract over the samples was removed.

The hASCs in the concentration of 1×10^5/ml were inoculated on a flat bottom 96-well plate and cultivated in a growth media with 2% fetal calf serum at 37 °C, 5% CO_2 and absolute humidity during 3 days. After 3 days of cultivation the growth media over the cells was substituted by the extract taken from the samples and a growth media with 2% fetal calf serum in the concentration 0:1; 1:0; 1:1; 1:2; 1:4; 1:8, correspondingly. After 24 hours of cultivation with the extract the MTT test was performed. Optical density was registered at 540 nm on a Sunrise analyzer (Austria).

1.12.2. Cytotoxicity assessment – direct contact method

Hydrogel cell-less scaffold samples were used, their diameter was 15 mm, thickness 2 mm (9 SE scaffold samples and 9 mSE scaffold samples). The samples were transferred to the hASCs culture and covered with 5 ml of full growth media. 24 hours before the beginning of the experiment the hASCs were inoculated on cultural dishes (Corning 60 mm × 15 mm; USA) with density being 20,000/cm^2. The hASCs with scaffold samples were cultivated in a CO_2 temperature regulated chamber under standard conditions. The control was hASCs culture cultivated under the same conditions and in the same concentration but without the samples. After control periods (24, 72, 144 hours) the condition of hASCs was assessed (morphology, density, viability). After the completion of the experiment (144 hours of cultivation with samples) the immunophenotype of the cells was determined (# 1.5).

1.12.3. Comparative study of 3D hASCs cultivation in SE and mSE scaffolds

In order to carry out the study SE (5 samples) and mSE (5 samples) were formed according to the method described in # 1.7, introducing into their content hASCs composite. The samples were placed into cultural dishes with full growth media and cultivated up to 6 days in a CO_2 temperature regulated chamber under standard conditions.

1.13. Cell distribution density characteristic in equivalents

In order to characterize cell distribution density in equivalents fragments the area of which was 0.64 cm^2 were separated from studied samples (SE – 5 samples, mSE – 5 samples) by means of a template. The number of cells was determined by counting the nuclei 24 hours after the beginning of forming SE and mSE. In order to achieve this the selected sample fragments were transferred to a 24 well plate "Black Visiplate^{T-M}TC" (Wallac Oy, Finland). Later analysis of the cell number was performed according to the quantitative analysis method for the cellular component of the scaffold (Pat. № 2675376 RF… dated 17.07.2017 [13]). The method includes intravital staining of the cell nuclei in the scaffold using Hoechst 3334 (USA), fluorescent microscopy with Z-stack function (Cytation 5 image tool with Gen 5 Imedge+ software; BioTek, USA). 5 microphotographs from each sample were analyzed which were taken from different viewpoints (enlargement: 4× lens, 10× eyepiece) at random sites in the thickness of the samples. Objects were registered at 530 μm sites along the Z axis. In order to obtain a characteristic of the cell distribution density in SE and mSE cross-linked Z-stack microphotographs were used with calculation of cell nuclei number and subsequent recalculation of cell number per 1 mm^3.

1.14. Statistical analysis

The results of the studies have been processed by distribution-free statistic methods using Mann–Whitney criteria and Wilcoxon pair comparisons, with the help of STATISTICA 6.0 programme package.

2. RESULTS

2.1. pASCs characteristics

After the cells were recovered from the adipose tissues their adhesion to plastic material was recorded in 24 hours. As a rule, by day 6–10 the cells formed a subconfluent monolayer (60–70%). After the formation of the subconfluent monolayer the cultures were subcultured. During the whole follow-up period cells obtained from porcine adipose tissue were morphologically uniform, had a typical fibroblast-like structure and spread out well on the plastic material. The cells were characterized by clear contours, pronounced projections which formed intercellular contacts. In the centers of the cells oval nuclei were noted with dense nucleoli.

In the course of evaluating the differentiation potential of cultivated cells obtained from porcine adipose tissue on day 6–7 after introducing a differentiating media the appearance of adipose vacuoles was noted in the cells which were stained by a specific Oil Red stain. Further cultivation in a differentiated media led to a further increase in the number of cells with adipose vacuoles

(Fig. 1, b). Osteogenic differentiation was indicated by the appearance of calcium salts in the cells which were stained by a specific stain – alizarin red (Fig. 1, d). On day 13–15 osteogenic differentiation was confirmed by osteocalcin staining of the cells obtained from adipose tissue. Formation of small spheroids (pellets) typical for chondrogenic differentiation was recorded in vitro already one day after adding DMEM/F12 media with differentiating additive. In these spheroids on day 6 type II collagen deposit was recorded by polyclonal antibodies (Fig. 1, f).

The cell culture phenotype was determined in the process of culture growth for passages 2 through 6. In the process of study it has been noted that the pan-leucocyte antigen CD 45 was not noted on the cells derived from adipose tissues at all the stages of the study (less than 1%). CD expression on the 2nd passage amounted to an average of 56%. In the process of culture growth the percentage of cells expressing CD 44 increased and reached 99.7% by passage 6. A typical MSCs marker CD 90 was expressed by about 75% of the cells at passage 2. In the process of growth the percentage of cells expressing CD 90 increased and amounted to about 90% by passage 6 (Fig. 2, a). The number of cells expressing CD10 was increased from passage to passage in all cultures and by passage 6 the number of cells expressing CD10 amounted to about 70%. Thus the phenotype of cells obtained from porcine fatty tissue may be described as CD 90+, CD 44+, CD 10+, CD 45–, which corresponds to the MSCs phenotype.

The phenotype of ASCs obtained from several animals (Fig. 2, b) and dynamic changes in the expression of the main markers were similar. Increased CD 44 and CD 90 expression was noted up to passage 3–4. At later periods the expression of these markers stayed at an invariably high level. Analysis of CD 10 expression showed that expression of this antigen increased continuously till passage 6. CD 45 expression by the cells remained at an invariably low level (below 1%).

2.2. Comparative study of the influence of hydrogel scaffolds on hASCs culture

Comparative study of the cytotoxicity of cell-less hydrogel scaffolds on the basis of human blood plasma

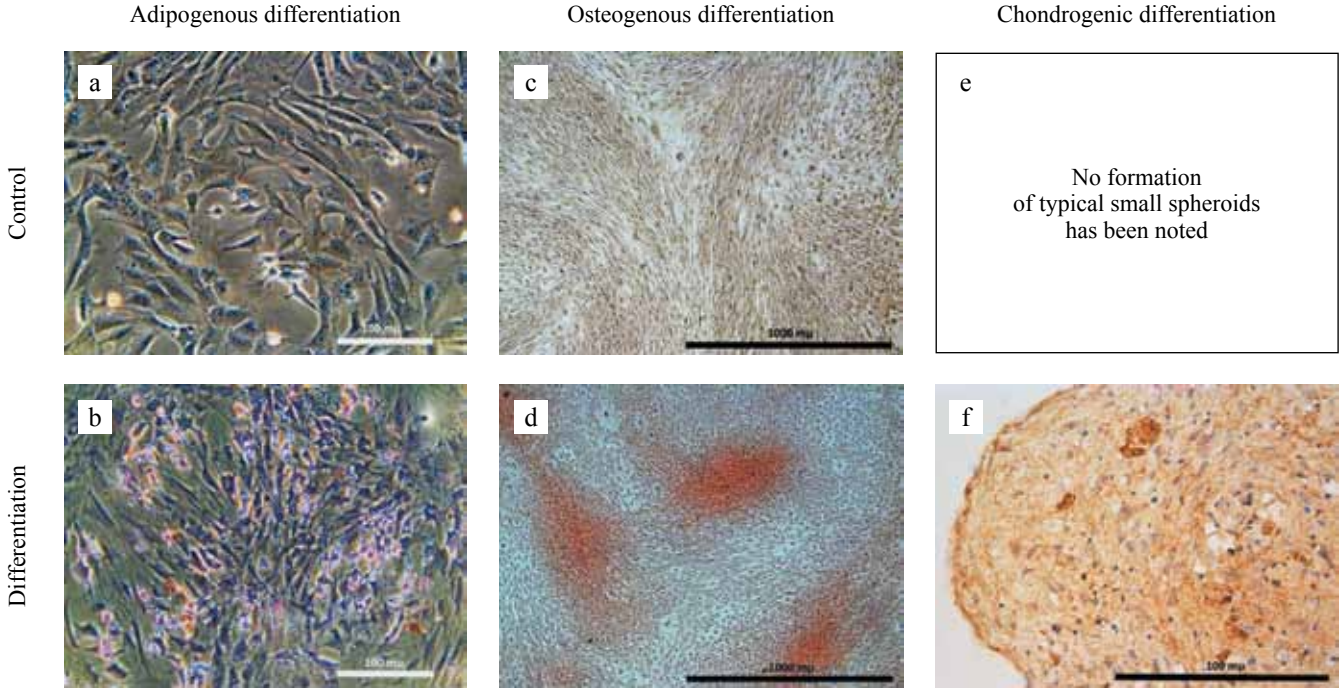

Fig. 1. Differentiation of pASCs. a, b – Adipogenous differentiation of pASCs, Oil Red staining, (cultivation day 15; objective lens 20×, eyepiece 10×; phase contrast): a – control – pASCs culture, third passage without use of differentiating media, the subconfluent monolayer is formed by typical fibroblast-like cells. No adipose vacuole formation has been noted in control culture cells; b – experimental pASCs culture after cultivation in a differentiating media. In the culture cells stained by a specific Oil Red stain adipose vacuoles are clearly noted which demonstrates the pASCs capability of adipogenous differentiation; c, d – Osteogenous pASCs differentiation. Osteogenous pASCs differentiation, staining by alizarin red (cultivation day 15; objective lens 5×, eyepiece 10×, light microscopy): c – pASCs control culture is presented in the form of a subconfluent monolayer formed by morphologically identical spindle-shaped cells, no calcium deposits noted; d – pASCs culture, experimental series after cultivation in a special differentiating media. The culture appears as a subconfluent monolayer. Calcium deposits are clearly visualized which are stained by a differentiating stain (alizarin red); f – pASCs chondrogenic differentiation (light microscopy). a – objective lens 5×, eyepiece 10×; b – Staining in a spheroid formed by pASCs culture after cultivation of type II collagen by polyclonal antibodies in a chondrogenic differentiated media (Abcam, ab34712; objective lens 40×, eyepiece 10×). Formation of small spheroids and type II deposition in them by the cells indicates chondrogenic differentiation of pASCs

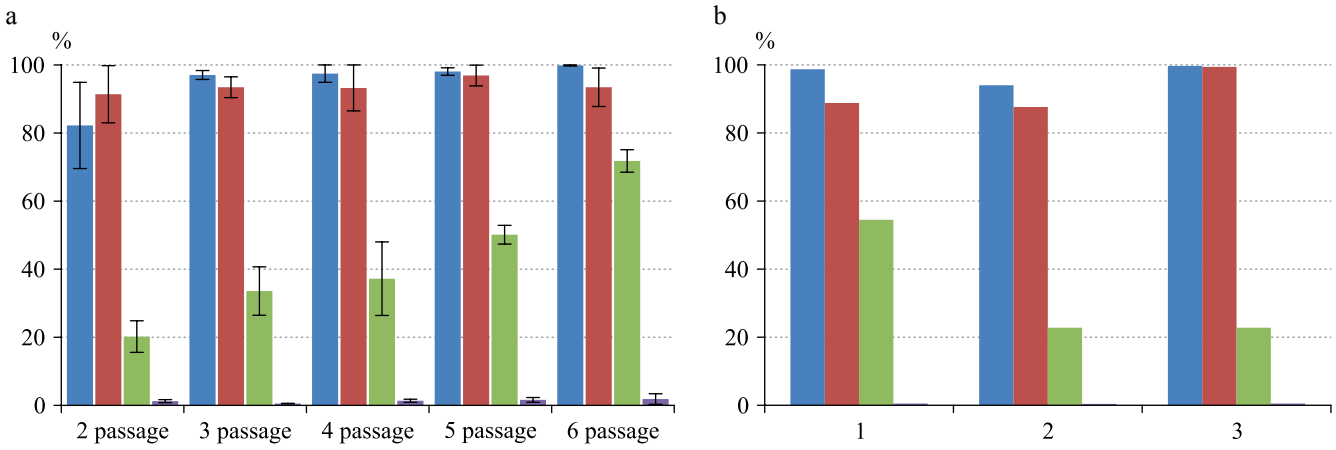

Fig. 2. Expression of differentiation markers for pASCs. (a) example of the changing of cell phenotype from one of the experimental animals in the course of cultivation. From passage to passage an increased number of cells is noted which express CD 44 (blue), CD 90 (red), CD 10 (green). The number of cells in the pASCs culture which express CD 45 (purple) was less than 1% during the whole cultivation period; (b) comparative diagram of MSCs phenotype obtained from the adipose tissue of three different animals (passage 3). In all the experimental cultures the number of cells expressing CD 44 (blue) exceeded 90%, CD 90 (red) – 87% and CD 10 (green) – 22%. Expression of the pan-leucocyte antigen CD 45 on the cells of the cultures under consideration amounted to less than 1%

cryoprecipitate (SE scaffold) and on the basis of porcine blood plasma cryoprecipitate (mSE scaffold) showed an absence of cytotoxic activity on human MSCs (Table 1). Analysis of MTT test results showed a pronounced stimulating action of the extract obtained from cell-less scaffolds under examination on the proliferative activity of hASCs. Thus, after exposure to the extract collected after the first day of incubation from SE scaffolds the number of metabolically active cells in the culture was increased by over 1.5 times as compared to the control group. A similar effect was noted after exposure to the extract from SE scaffolds after 8 days of incubation. After exposure to the extract collected from mSE scaffolds (days 1 and 8 of incubation) the number of metabolically active cells was increased by over 1.8 times as compared to the control group. Rarefaction of the extracts led to a decrease of their stimulating effect. At the same time, even when the extracts were diluted by half there was a statistically significant increase in the number of metabolically active cells as compared to the control group. During comparison of the action of extracts from cell-less SE and mSE scaffolds on the hASCs culture no significant differences according to the results of the MTT test have been obtained (Table 1).

In the course of human hASCs cultivation in the presence of cell-less hydrogel SE and mSE scaffolds no negative effect of the scaffolds on the hASCs culture has been noted. There was no statistically significant difference between cell density in the culture and their absolute number in the cultural dishes under investigation (SE scaffold, mSE scaffold) and the control group at all times during the study (Table 2). The cells in experimental cultural dishes and control dishes were well spread out on the plastic material and formed a subconfluent level. The hASCs were morphologically homogeneous and had a characteristic fibroblast-like form with clear contours, pronounced projections which formed intercellular contacts. All the cells were characterized by high viability (Table 2). The phenotype of the cells from control and experimental cultural dishes did not vary and corresponded to the MSCs phenotype. Cells expressed CD 90, CD 105, CD 73, CD 44, CD 10 and did not express CD 45, CD 34, CD 14, CD HLA DR.

Human adipose tissue hASCs incapsulated in SE and mSE scaffolds were characterized by equal activity in the

Table 1

Cytotoxicity study of cell-less hydrogel scaffolds

Extract withdrawal	Scaffold	Control 0:1	Extract 1:0	Extract 1:1	Extract 1:2	Extract 1:4	Extract 1:8
Day 1	SE	0.341 ± 0.007	0.518 ± 0.011*	0.537 ± 0.026*	0.442 ± 0.020*	0.423 ± 0.020*	0.334 ± 0.007
	mSE	0.322 ± 0.011	0.568 ± 0.026*	0.442 ± 0.029*	0.403 ± 0.014*	0.370 ± 0.011*	0.393 ± 0.016*
Day 8	SE	0.327 ± 0.010	0.519 ± 0.021*	0.512 ± 0.014*	0.437 ± 0.011*	0.371 ± 0.020	0.348 ± 0.022
	mSE	0.282 ± 0.006	0.557 ± 0.032*	0.505 ± 0.025*	0.405 ± 0.022*	0.364 ± 0.016*	0.323 ± 0.017

Note. * – $p < 0.05$, comparison vs control, Wilcoxon criterion; ■ – $p < 0.05$, comparison of SE vs mSE, Mann–Whitney criterion.

course of 3D cultivation. Thus, already after 24 hours the cells demonstrated active matrix-cell adhesion and began sprouting cellular projections (Fig. 3, a, b). In the process of further cultivation dynamic 3D cell growth was noted with formation of multiple projections and intercellular contacts. On day 6 formation and development of cellular

Table 2

Comparative study of the influence of cell-less model skin equivalent on hASCs culture

Cultivation time	Cell density ($\times 10^3/cm^2$)	Absolute number of cells ($\times 10^3$)	Cell viability (% of viable cells)
	Control		
24 hours	26.73 ± 1.17	534.67 ± 23.33	98.70 ± 0.15
72 hours	39.47 ± 1.81	789.48 ± 36.15	99.52 ± 0.18
144 hours	40.64 ± 0.95	812.81 ± 18.94	99.58 ± 0.10
	SE scaffold		
24 hours	27.894 ± 0.90	557.89 ± 17.97	99.25 ± 0.31
72 hours	39.29 ± 1.47	785.810 ± 29.30	99.55 ± 0.18
144 hours	45.17 ± 2.34	902.35 ± 46.95	99.63 ± 0.12
	mSE scaffold		
24 hours	27.96 ± 1.05	559.11 ± 21.03	99.77 ± 0.15
72 hours	38.68 ± 2.36	773.59 ± 47.14	99.13 ± 0.10
144 hours	42.51 ± 1.97	850.12 ± 39.34	99.65 ± 0.16

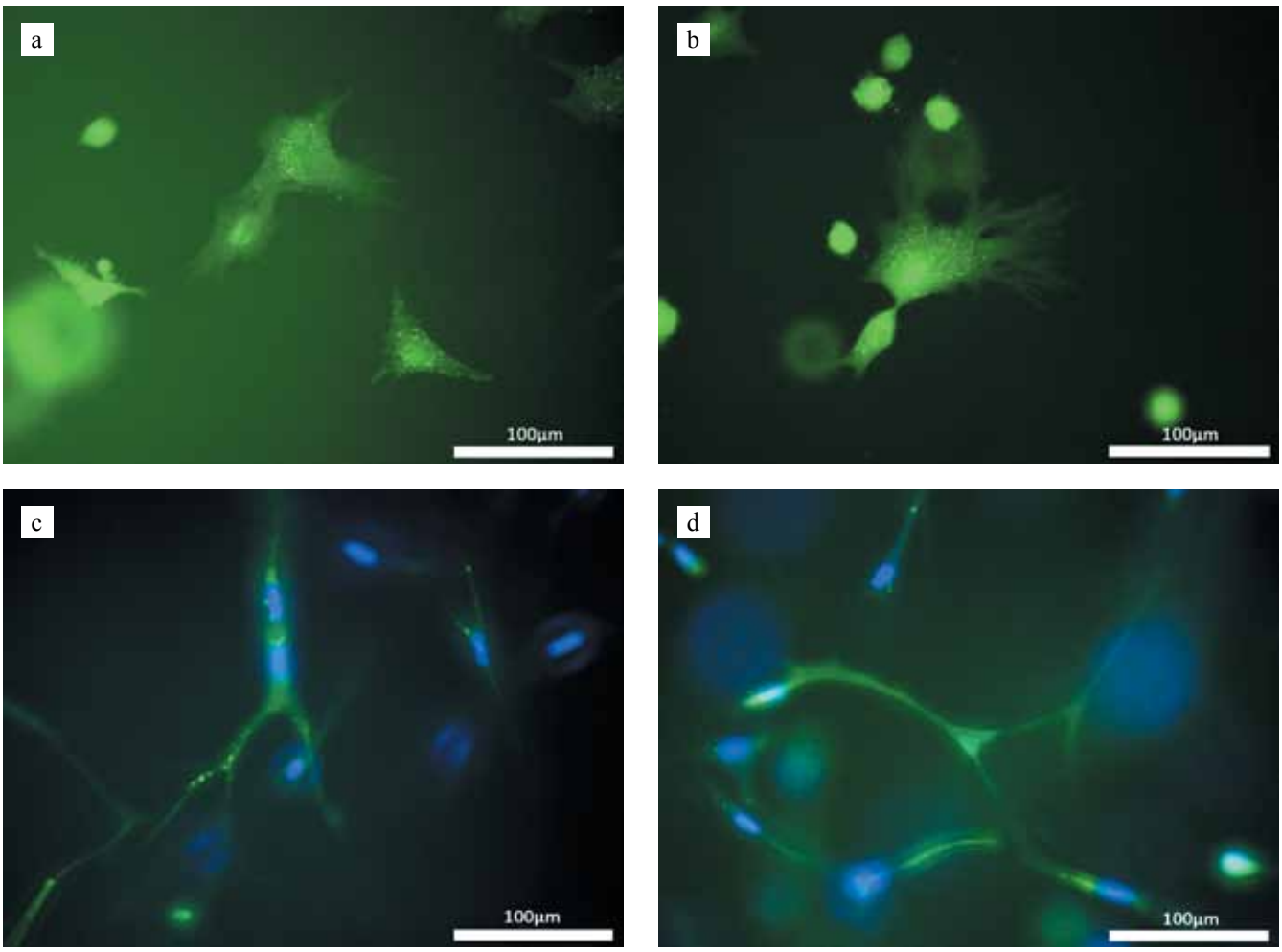

Fig. 3. hASCs cultivation in SE (a, c) and scaffold on the basis of porcine blood plasma cryoprecipitate (b, d). Cellular events in the course of hASCs cultivation in SE and in the scaffold on the basis of porcine blood plasma cryoprecipitate had a similar character: a, b – 24 hours of cultivation: the cells are spread out, sprout cellular projections; c, d – day 6 of cultivation: 3D cell growth with formation of multiple projections and intercellular contacts. Fluorescent microscopy: viable cells are stained with the specific fluorescent Calcein AM stain (green); b, d – cell nuclei (blue) are stained with Hoechst 3334 fluorochrome. *Note:* cells and nuclei lying in other layers of the 3D SE and mSE scaffold structure are out of focus (related to the layer where the cells are in focus)

networks was noted in SE and mSE scaffolds (Fig. 3, c, d). Viability of the cells cultivated in SE and mSE scaffolds was confirmed by staining with Calcein AM.

2.3. Comparative analysis of SE and mSE

Model skin equivalents formed on the basis of porcine blood plasma cryoprecipitate with pACSs possessed characteristics similar to those in skin equivalents formed on the basis of human blood plasma cryoprecipitate and hASCs. SE and mSE had a form-stable, elastic, transparent hydrogel structure (Fig. 4, a, b). The cells were spread uniformly along the whole structure of the equivalents, both in SE and in mSE (Fig. 4, c, d). There was no statistically significant difference between the number of cells detected on 1 mm^3 24 hours after the formation of the equivalents in SE and mSE, which amounted to 135.70 ± 24.78 and 124.93 ± 22.81 correspondingly.

In the process of SE and mSE cultivation no significant differences have been detected in the development of cellular events. 24 hours after the formation of the equivalents matrix-cell adhesion was noted, cellular projection formation and the spreading out of the cells started. In the process of further cultivation human adipose tissue MSCs and porcine adipose tissue MSCs demonstrated active 3D growth (Fig. 4, e, f). The cells formed multiple projections in different directions and cellular contacts which later led to the formation of a cellular network.

3. DISCUSSION

The development of biomedical cell products inevitably involves the stage of preclinical studies using laboratory animals. At the same time studies which involve the use of large laboratory animals is a less common practice than the practice of studies using small laboratory animals, such as rodents. Therefore while preparing study protocols it is not always possible to be guided by similar works and fully utilize standard or generally accepted methods.

In order to develop a comprehensive approach to forming a BMCP model corresponding to the 'homologous drug' strategy for preclinical studies on a large laboratory animal (pig) skin equivalent was chosen as the object of study. The main components of the skin equivalent presented in this work are human blood plasma cryoprecipitate, type I cod collagen, bovine thrombin and human adipose tissue mesenchymal stem cells. Blood plasma cryoprecipitate and hACSs as components of SE are in allogenous conditions related to the recipient (human). When SE are used in studies on a pig model these components will change from allogenous conditions to xenogenous as related to the model recipient (pig). It is known that the same cell products and tissue engineered products may have a significantly higher immunogenic activity under xenogenous conditions as compared to allogenous. In a number of experimental studies it has been shown that the results of using xenogenous MSCs are significantly inferior to the results obtained while using allogenous or autogenous cells and may lead to the development of negative effects. For example, P. Niemeyer et al. (2010) have found that in the groups of animals who received treatment with xenogenous MSCs the results of bone tissue regeneration were worse by all the parameters considered compared to the group of animals who received treatment with autogenous MSCs [14]. In order to avoid misrepresentation of results related to species-specific immune response in the course of future preclinical studies we have developed a model skin equivalent for preclinical studies on pigs. Based on the correspondence to the 'homologous drug' strategy while developing a model skin equivalent as part of SE in the course of its formation it was necessary to substitute the human blood plasma cryoprecipitate and hASCs by porcine blood plasma cryoprecipitate and pACSs. Sea collagen and bovine thrombin which are included in the composition of SE did not require substitution in our opinion due to the fact that they are xenogenous components both for humans and for pigs.

Having analyzed literature sources we did not find any generally accepted protocol for isolation, cultivation or assessment of pASCs. We have prepared and tested relatively simple protocols which are applicable for experimental work. Their fundamental principles are described in ## 1.6–1.9 of the 'Materials and methods' section. The presented approaches enable to obtain biomaterial from a specific area of adipose tissue, which enables to get uniform primary material and standardize the tissue recovery technique. Using a dermatome enables to obtain adipose tissue grafts from a certain depth. The thickness of the grafts (up to 1.2 mm) simplifies the primary material processing as no coarse primary mechanic homogenization or additional procedures aimed at forming thin grafts are required before the mechanical disaggregation stage, as, for example, in the recovery of large volumes of adipose tissue [15].

The selected approaches enabled to perform a characteristic description of the cells obtained from porcine adipose tissue according to the main parameters required for their identification and use in experimental work: viability, morphology, differentiation potential, immunophenotype. The data received by us regarding isolation and description of MSCs from porcine adipose tissue, same as those found in literature sources [16, 17], demonstrate the capacity of pASCs for osteogenic, adipogenic and chondrogenic differentiation, manifestation of typical morphological characteristics and a certain phenotype.

One of the main components of the SE presented in the work is blood plasma cryoprecipitate. In the model SE human blood plasma cryoprecipitate was substituted by porcine blood plasma cryoprecipitate. The procedures

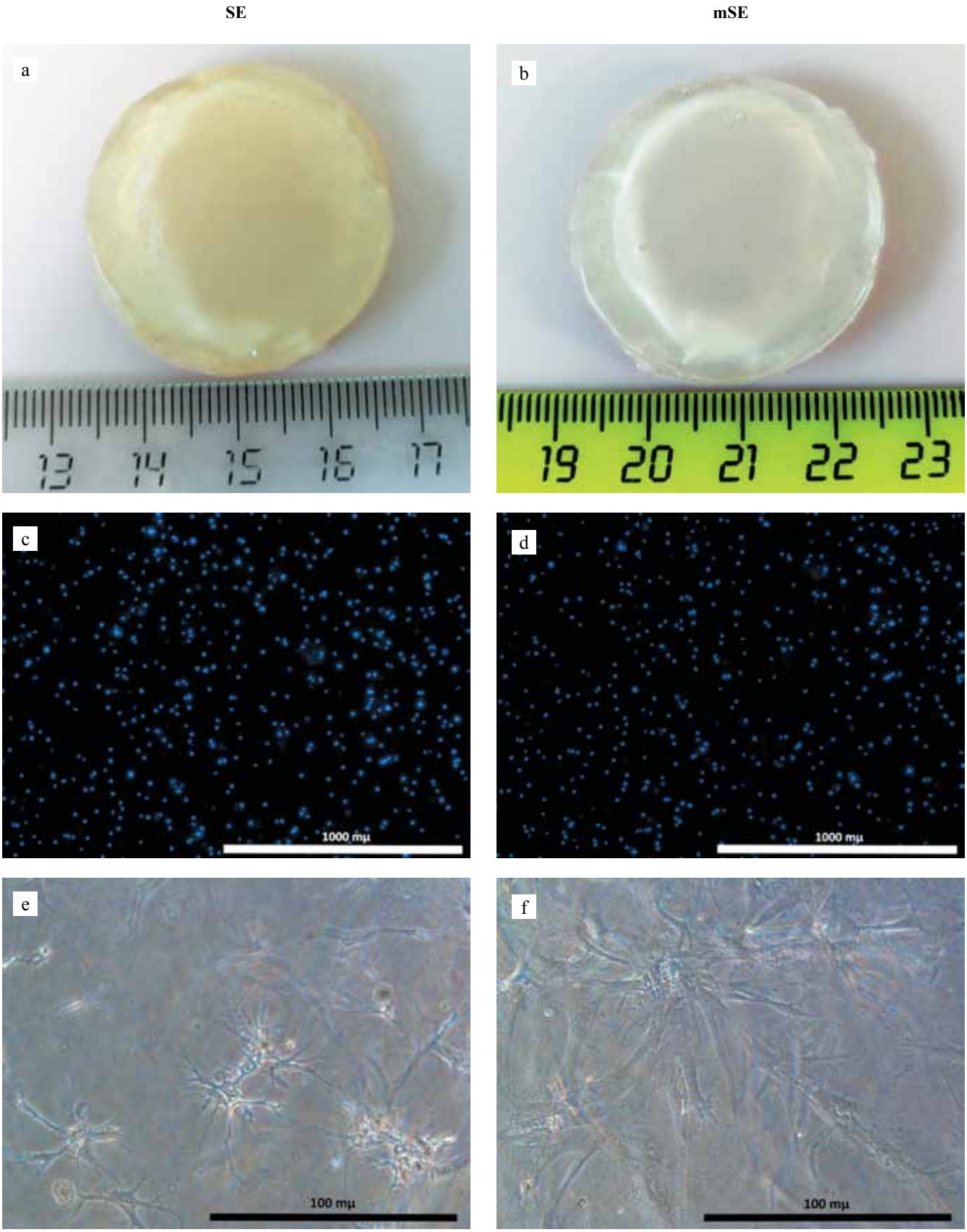

Fig. 4. SE and mSE: a, b – external appearance of the equivalents; c, d – cell nuclei, fluorescent microscopy: crosslinked microphotographs Z-stack (530 mμ), cell nuclei (blue) stained with Hoechst 3334 specific fluorochrome; e, f – MSCs cultivated in equivalents (day 3 of cultivation; phase contrast, objective lens 40×, eyepiece 10×)

related to obtaining porcine blood plasma and preparing cryoprecipitate technologically fully corresponded to those which were used in the process of preparing human blood plasma cryoprecipitate for SE formation. The basis of the SE formation method is the enzyme hydrolysis reaction which takes place in the presence of thrombin. The main structure-forming protein for the forming of the SE scaffold is blood plasma cryoprecipitate fibrinogen, the amount of which in the composite exceeds the amount of collagen by over 22 times. Thus the process of SE forming imitates the process of blood coagulation. The porcine blood coagulation system does not have any essential difference from the human blood coagulation system [18]. Based on this we used the same method for forming mSE that was used for forming SE. The possibility of using mSE in preclinical studies is based on the assumption that mSE should not have any significant differences in their properties as compared to SE. In order to check this assumption a comparative assessment of cytotoxicity and influence on hASCs culture was performed for cell-less SE and mSE scaffolds which were serving as scaffold cell carriers. Data of the MTT test confirmed the absence of cytotoxicity for mSE scaffolds. The results of the MTT test showed that the number of metabolically active cells in the presence of SE and mSE scaffold extracts grew significantly. This data indicates an increase in the cell proliferative activity in the presence of scaffolds. Both scaffolds are formed on the basis of blood plasma cryoprecipitate. Human blood plasma and porcine blood plasma contain quite many biologically active substances (amino acids, proteins, microelements, etc) [19]. It is well known that the concentration of many biologically active substances (BAS) in blood plasma cryoprecipitate is much higher that in blood plasma [20]. Blood plasma components may take active part in cell growth regulation. Thus, for example, calcium cations are present in blood plasma. M.N. Lee et al. have shown that increased Ca^{2+} concentration in the cultural medial leads to increased MSCs proliferative activity [21]. Also exogenous calcium which is included in the thrombin-calcium mixture used in scaffold forming is present in the sample scaffolds of the studied equivalents. Therefore the noted stimulating effect is most probably related to the action of biologically active substances extracted from scaffolds.

The direct contact test results confirmed absence of a negative influence on behalf of SE and mSE scaffolds on the viability, adhesion, morphology, proliferation and immunophenotype of hASCs. At the same time the results of this test did not show any stimulating action on behalf of cell-less scaffolds on the proliferation of hASCs. Most probably this is due to low concentration of BAS which are washed out from the scaffolds in this experiment. It is known that during cell cultivation the presence and concentration of BAS in the cultural media largely determines cultural growth [22]. Thus, in the course of the MTT test the extract was collected from 33 mm scaffolds which were incubated in 6 ml of the media. In the course of the biocompatibility test 15 mm sized scaffold samples and 5 ml of the media were used. At the same time the amount of cells impacted by BAS released by the samples in the MTT test was much lower as compared to the number of cells in the direct contact test. Therefore the results of the direct contact test may to a certain degree be compared to the results of the MTT test with highly diluted extract (1:8). In case of high dilution of the extract the number of metabolically active cells varied little from the control, and the results of the extract's influence on the cells were comparable with those obtained after cell cultivation in a growth media.

It is known that mesenchymal stem cells encapsulated into hydrogel scaffolds demonstrate 3D growth [23]. In order to determine to which extent an mSE scaffold ensures 3D cell growth and how comparable will cell events be in mSE and in SE the same hASCs cultures were encapsulated into both scaffold types. hASCs encapsulated into scaffolds supported their vital activity and demonstrated 3D growth. Both scaffolds are hydrogels. It is known that the hydrophilic nature of hydrogels facilitates the exchange of nutrients and metabolic products between the cells within the 3D structure, thus supporting high viability of the cells [24]. Cellular events noted in SE and mSE scaffolds did not show any significant differences throughout the observation period. It is known that viability, growth, morphology, migration, proliferative activity, etc. are largely determined by the scaffold content and structure [25]. The latter enables to suggest that the content and structure of SE and mSE scaffolds which influence cellular behaviour are comparable.

The latter statement is also confirmed by data obtained in the course of SE and mSE formation. Thus, it is known that the properties of a scaffold as a mechanical supporting structure for the cells are determined by its inner architectonics. In the course of SE and mSE formation the cells were distributed relatively uniformly throughout the whole structure of the equivalents with equal density. The SE and mSE structures are formed primarily by the fibrin network. Self-assembly of the fibrin fibers depends on the concentration of fibrinogen, thrombin and calcium [26]. Literature data bears evidence that protein pegylation also has a significant influence on the forming fibrin fibers [27]. The composite used to form scaffolds also includes collagen. Collagen of various origins is used quite extensively in scaffold technologies and has shown advantages as a structure-forming biopolymer for the formation of mono- and polycomposite scaffolds [28, 29]. Thus, the collagen used to form equivalents may also take part in forming SE and mSE structures. At the same time, concentrations and proportions of the abovelisted components in the course of developing mSE were the same as the ones used for forming SE. 3D cultivation of pASCs as part

of mSE confirmed the comparability of cellular events developing in mSE and in SE.

Summarizing the obtained results we can make a conclusion that the conditions of forming equivalents (content, proportions and concentrations of the main components which take part in structure formation, methodological approach) enable to obtain structures which provide similar mechanical support of the cells and allow to create comparable conditions for placement and interaction of MSCs.

4. CONCLUSION

A comprehensive approach has been presented for the development of a biomedical cellular product model on the example of a skin equivalent to be used in preclinical studies on a large laboratory animal, taking into account the 'homologous drug' strategy. In the course of preparing the model BMCP the components of SE (developed to be used in humans) which change from allogenous conditions to xenogenous in the course of transplantation to an animal were substituted. Compliance of the characteristics of cells obtained from porcine adipose tissues to requirements for MSCs has been confirmed. In the future the set of methods and approaches used in the work for obtaining, cultivating and characterizing pASCs, as well as obtaining porcine plasma, may be useful while carrying out work based on using animal cells, for example in the course of developing protocols for xenotransplantation of cells and cell-based constructions. Lack of cytotoxicity has been confirmed for the mSE cell-less scaffold. It has been proved that the mSE scaffold provides similar conditions for cell placement and 3D growth as the SE scaffold. Cellular events developing in the course of mSE and SE cultivation were also comparable. Therefore, mSE on the basis of porcine blood plasma cryoprecipitate and pASCs can be used for conduction of studies on large laboratory animals (pigs) as a model corresponding to the 'homologous drug' strategy. The authors hope that the set of methods and the protocol of work used in the development of this model equivalent will be useful in the course of preparing and carrying out preclinical studies of similar biomedical cell products.

The authors declare no conflict of interest.

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FORMATION OF EYEBALL ORBITAL STUMP USING TITANIUM NICKELIDE TISSUE-ENGINEERED CONSTRUCT AND AUTOLOGOUS BLOOD MONONUCLEAR LEUKOCYTES

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Objective: to study the morphological features of formation of the eyeball orbital stump using a titanium nickelide tissue-engineered construct and a suspension of autologous blood mononuclear leukocytes *in vivo*. **Materials and methods.** Experiments were performed on 54 sexually mature Wistar rats weighing 200–250 g. The animals were divided into 3 groups, depending on type of surgical intervention: group 1 (n = 18) consisted of animals in which eyeball orbital stump was formed after evisceroenucleation through implantation of a titanium nickelide tissue-engineered construct and a suspension of autologous blood mononuclear leukocytes in the scleral sac; group 2 (n = 18) – the eyeball orbital stump was formed through implantation of titanium nickelide tissue-engineered construct in the scleral sac; group 3 (n = 18) – orbital stump was formed using an Alloplant implant. **Results.** It was established that in group 1 rats, on day 7 following surgery, the specific volume of connective tissue was 7.9 times ($p_U = 0.048$) higher than in group 2 rats and 15.8 times ($p_U = 0.039$) higher than in group 3 rats. On day 14 after surgery, the volume of connective tissue in the eyeball orbital stump of group 1 rats reached the highest value compared to that in the other groups. The numerical density of newly formed vessels in the eyeball orbital stump of group 1 rats, starting from day 14 after surgery up to the end of experiment (day 21), was statistically significantly higher than that in the other groups. Moreover, on day 21, this indicator was 4.0 times ($p_U = 0.001$) higher in group 1 rats than in group 2 rats and 9.8 times ($p_U = 0,0003$) higher than in group 3 rats. **Conclusion.** Implantation of titanium nickelide tissue-engineered construct and a suspension of autologous blood mononuclear leukocytes into the scleral sac after evisceroenucleation in an *in vivo* experiment leads to accelerated maturation of the connective tissue and intensive vascularization in the eyeball orbital stump. This ensures strong fixation of the implant and reduces risk of rejection.

Keywords: blood mononuclear leukocytes, eyeball orbital stump, tissue-engineered construct, titanium nickelide, cell technology.

INTRODUCTION

Due to the achievements of up-to-date ophthalmology, the treatment of various serious diseases of the visual organ is becoming more effective. However, despite all the ongoing therapy measures, it is impossible to save the eyeball as an organ [1, 2]. It should be noted that about 75% of enucleations are performed without the formation of a musculoskeletal stump and implantation of an orbital insert [2, 3]. This, in turn, leads to the development of anophthalmic syndrome. The clinical pattern of this complication is featured with the sunken orbital-palpebral sulcus, eyelid deformity, ptosis, and an incomplete closure of the palpebral fissure [1, 4]. Treatment of anophthalmic syndrome is a time-consuming and multi-stage process aimed at restoring the volume of the conjunctival cavity and other anatomical structures, as well as restoring the orbital tissue volume deficiency through implanting an inert, biocompatible material [1, 3, 5]. Several materials are offered for use as an orbital implant. Some of them, cartilage, hydroxyapatite, and carbon composites are widely used in orbital surgery, others, e.g. tantalum, ceramics, injection hydrogel, monolithic silicone have limited use for their high cost and high adverse event rate [1, 3, 5].

At present, ophthalmic surgeons show increasing interest in porous materials with the structure providing a sufficiently rapid ingrowth of surrounding tissues, thus contributing to the strong fixation of the implant in orbit. Still, when using porous materials, especially in the long term, such complications as the implant exposure, its infection and rejection cannot be excluded. A possible solution to the problem is the use of cell technology during orbital implantation [6–12].

Purpose: to study the morphological features of formation of the eyeball orbital stump using a titanium nickelide tissue-engineered construct and a suspension of autologous blood mononuclear leukocytes *in vivo*.

MATERIALS AND METHODS

Experiments were performed on 54 sexually mature Wistar rats weighing 200–250 g (54 eyes).

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Experiments were performed on 54 sexually mature Wistar rats weighing 200–250 g (54 eyes) from the vivarium of the FGBOU VO SibGMU (Siberian State Medical University) of the Ministry of Health of Russia. Before the experiment, all animals were quarantined for a week under vivarium conditions in the usual food regime. The experimental studies protocol was approved by the local ethics committee of the GOU VPO SibGMU of Roszdrav (Russian Federal Service on Surveillance in Healthcare) of November 29, 2010, Reg.No.1715.

The animals were divided into 3 groups, depending on type of surgical intervention: group 1 (n = 18) consisted of animals in which eyeball orbital stump was formed after evisceroenucleation through implantation of a titanium nickelide tissue-engineered construct and a suspension of autologous blood mononuclear leukocytes in the scleral sac; group 2 (n = 18) included rats with the eyeball orbital stump formed through implantation of titanium nickelide tissue-engineered construct in the scleral sac; group 3 (n = 18) consisted of animals with the orbital stump formed using an Alloplant implant.

The titanium nickelide implant is made of porous titanium nickelide filament TN-10 of 100 μm thickness (Certificate of conformity No. РОСС RU ЛЯ79НО93 37 of 15.04.2011) [13], rounded, 4–5 mm in diameter. The implant is produced in the facilities of the Scientific Research Institute of Medical Materials (supervised by Professor V.E. Gyunter, Doctor of Technical Sciences).

The Alloplant biomaterial implant is manufactured by the FGU VTsGiPKh (Russian Center for Ophthalmic and Plastic Surgery of the Ministry of Health of Russia, Ufa, Russian Federation) of subcutaneous fat of human sole, rounded, 5 mm in diameter.

Mononuclear leukocytes from the experimental animals' blood were isolated immediately prior to surgery by fractionation on a ficoll-verographin separation solution (1.067–1.077 g/ml density). The cells purity was 96–98%, the percentage of stained (dead) cells was 1.5–2%, not exceeding the allowed (no more than 3%) limit.

Under operating conditions with etherization, all animals of 3 groups (54 rats) underwent evisceroenucleation of an eye, followed by placement of the corresponding implant in the scleral sac of the eyeball. Group 1 animals (18 rats) were injected with 0.1 ml of a freshly isolated autologous mononuclear blood leukocytes suspension (cell density: 200 thousand cells / 1 ml) in the structure of the titanium nickelide implant. All animals of 3 groups were postoperatively instilled with Tobramycin solution (6 times a day) in the conjunctival cavity of the operated eye.

The experiment lasted 21 days. During the experiment, on the 1st, 3rd, 7th, 14th, 21st days after the operation, an external examination was made, biomicroscopy of the operated eyes to assess the condition of the palpebral conjunctiva and the eyeball stump, of the edges of the surgical wound and sutures, as well as the presence and nature of the discharge in the conjunctival cavities.

On days 7, 14, and 21 after surgery, six rats from each group were removed from the experiment with the operated eyeball stumps removed, the obtained material fixed and hematoxylin-eosin stained by van Gieson for 200-, 300- and 400x light microscopy. At all stages of the experiment, the experimental animals were euthanized in compliance with the EU rules and norms (86/609EEC) and Helsinki Declaration.

Data processing

In slices morphometry with the ImageJ 1.50i software, mono- and polynuclear leukocytes, plasmocytes were counted, the volume of the stroma and the numerical percentage density of the newly formed vessels were determined. The results were statistically analyzed with the IBM SPSS Statistics 20 statistical package. The normality of distribution of indicators was checked by Kolmogorov–Smirnov test. The variables with a normal distribution were analyzed with Student's t-test. The results are presented in the M ± m form, where M is the arithmetic mean value, m is SEM. If the data distribution did not correspond to the normal distribution law, a nonparametric criterion was used, Mann–Whitney test (p_U). Differences were considered statistically significant at $p < 0.05$.

RESULTS

One day after the operation, the external examination of the animals of all 3 groups (54 rats) showed moderate edema and hyperemia of the eyelid's conjunctiva and the operated eyeball stump, which gradually decreased by the 3rd to the 4th days. The formed eyeball stump in all experimental rats (100%) was rounded and moderately mobile. Biomicroscopy showed adapted edges of the surgical wound in the suture area, and a small amount of mucous discharge was found in the conjunctival cavity of the operated eyes in rats. Throughout the experiment (21 days), no cases of exposure or rejection of an implant placed in the scleral sac of the operated eyeball were revealed in animals of all 3 groups.

According to light microscopy, in animals of the group 1 (6 rats), on the 7th day after evisceroenucleation and implantation of a tissue-engineering titanium nickelide construct and a suspension of autologous mononuclear blood leukocytes in the eyeball stump, extensive accumulations were found in the scleral sac of the mononuclear leukocytes (6259.0 ± 1646.0 cells per 1 mm^2 section) (Fig. 1), a small number of plasmocytes (443.6 ± 200.5 cells per 1 mm^2 section) and single polymorphonuclear leukocytes (PML) (344.9 ± 165.1 cells per 1 mm^2 section). Around the titanium nickelide implant, multiple focal accumulations of immature fibroblasts were detected. Thin collagen fibers were moderately edematous,

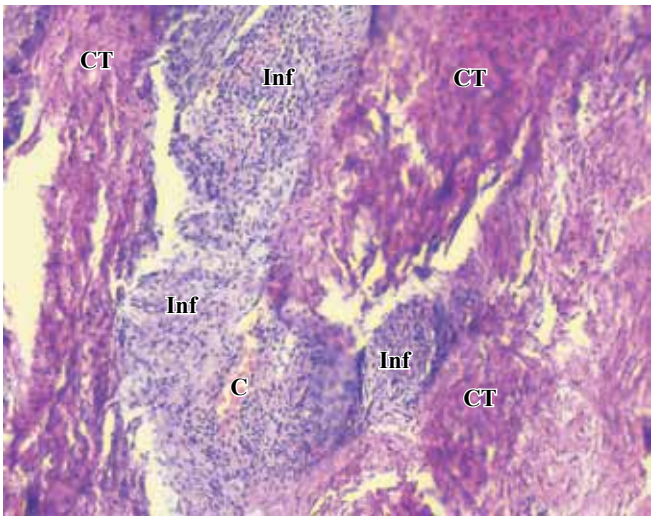

Fig. 1. Growth of loose fibrous connective tissue, focal mononuclear infiltration and newly formed vessels in the orbital stump of the 1st group of animals on the 7th day after evisceroenucleation with implantation of a tissue-engineering construction from titanium nickelide and suspension of autologous blood mononuclear cells. CT – connective tissue, Inf – cell infiltration, C – vessels. Stained with hematoxylin and eosin. ×400

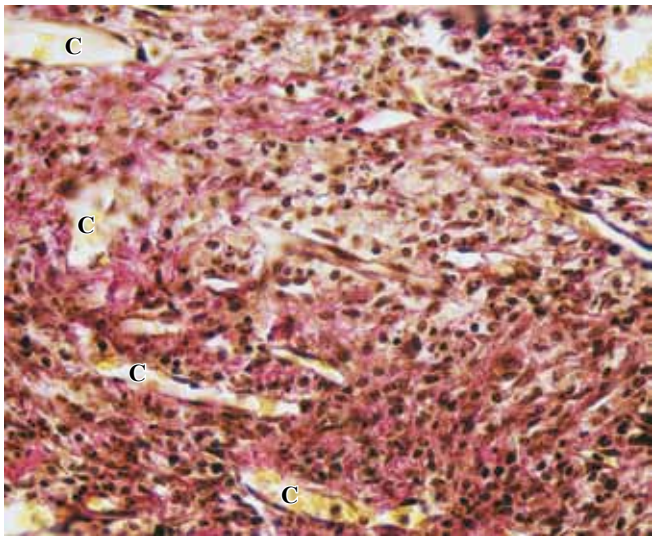

Fig. 2. Diffuse lymphocytic leukocyte infiltration and single newly formed vessels in the orbital stump of the 2nd group of animals on the 7th day after evisceroenucleation with implantation of titanium nickelide. C – vessels. Stained with hematoxylin and pikrofuksin by the method of Van-Giezon. ×300

Table

Quantitative ratio of stroma and newly formed vessels in 1 mm² section of the eyeball stump in animals, depending on the type of implant, M ± m, p_U

Experiment days	Stroma volume, % (n = 10)			Blood vessels numerical density, % (n = 10)		
	Group 1	Group 2	Group 3	Group 1	Group 2	Group 3
Day 7	0.158 ± 0.1	0.02 ± 0.01	0.01 ± 0.005	0.04 ± 0.027	0.02 ± 0.014	0.01 ± 0.006
Day 14	95.0 ± 2.1 p_1 = 0.0002; p_2 = 0.0001	0.3 ± 0.14	0.2 ± 0.11	5.0 ± 2.1 p_1 = 0.04; p_2 = 0.03	0.04 ± 0.03	0.02 ± 0.01
Day 21	79.1 ± 3.4 p_2 = 0.0005	94.7 ± 1.9	5.3 ± 1.9	21.6 ± 3.1 p_1 = 0.001; p_2 = 0.0003	5.3 ± 1.9	2.2 ± 1.2

Note. p_1 – significance level of differences compared with the data of group 2; p_2 – significance level of differences compared with the data of group 3; M – sampling mean, m – error of mean.

loosely located. Newly formed vessels were found between the fibers (Table, Fig. 1).

In animals of the group 2 (6 rats) on the 7th day after evisceroenucleation and titanium nickelide construct implantation in the scleral sac in the cavity of the eyeball stump, diffuse, uniform lymphocytic leukocyte infiltration (1916.6 ± 495.3 cells per 1 mm² section), a small number of immature fibroblasts, and edematous collagen fibers loosely located around the implant were detected. Among the fibers, single newly formed vessels were revealed (Table, Fig. 2).

In animals of the group 3 (6 rats), on the 7th day after evisceroenucleation and Alloplant biomaterial construct implantation in the scleral sac, the cavity of the eyeball stump was filled with adipose tissue interrupted by single mononuclear leukocytes (111, 5 ± 41.8 cells per 1 mm² section), a small PML amount (9.6 ± 4.8 cells in 1 mm² section), thin collagen fibers (Fig. 3) and single newly formed vessels were found (Table).

On the 14th day after the operation, in animals of group 1 (6 rats), there was an extensive proliferation of fibrous connective tissue in the cavity of the eyeball stump. Collagen fibers were arranged more orderly than on the 7th day. Accumulations of mononuclear leukocytes were detected between the fibers (9093.8 ± 891.0 cells per 1 mm² section). Around the titanium nickelide implant, newly formed vessels were found (Table), most of which began to differentiate into arterioles and venules.

In animals of group 2 (6 rats), on the 14th day after the operation, in the cavity of the eyeball stump, there was an overgrowth of loose fibrous connective tissue with significant edema and moderate lymphocytic macropha-

ge infiltration (4744.0 ± 928.0 cells per 1 mm^2 section). Thin-walled capillaries were found between collagen fibers, single arterioles and venules appeared (Table).

In animals of group 3 (6 rats), on the 14th day after the operation, in the cavity of the eyeball stump there was a slight lymphocytic macrophage infiltration (103.6 ± 49.4 cells per 1 mm^2 section) and the proliferation of thin collagen fibers between the fat segments of the Alloplant biomaterial implant. Around the implant, single newly formed vessels were found (Table).

On the 21st day after the operation, mature connective tissue was found in the animals of group 1 (6 rats) in the cavity of the eyeball stump. Thick bundles of collagen fibers were arranged compactly and orderly (Fig. 4). Small focal accumulations of mononuclear leukocytes

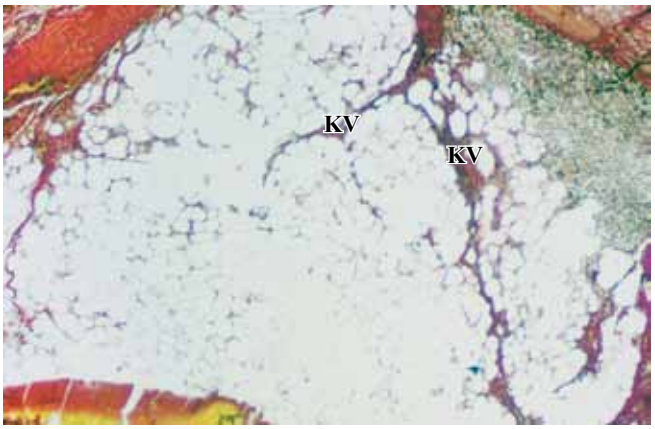

Fig. 3. Thin, single collagen fibers between the segments of adipose tissue in the orbital stump of the 3rd group of animals on the 7th day after evisceroenucleation with implantation of biomaterial "Alloplant". KV – collagen fibers. Stained with hematoxylin and eosin. ×200

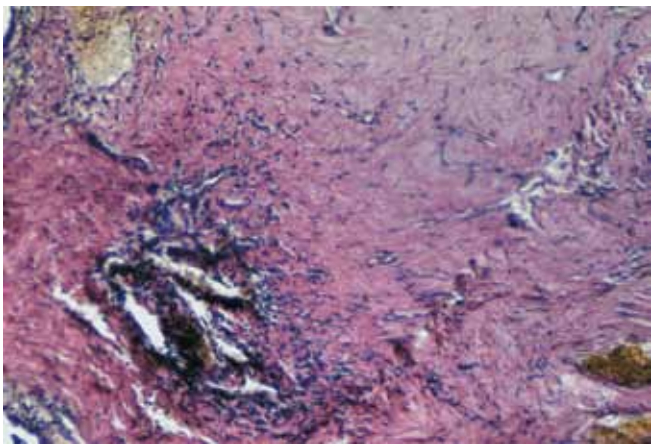

Fig. 4. Orderly arranged bundles of collagen fibers in the orbital stump of the 1st group of animals on the 21st day after evisceroenucleation with implantation of a tissue-engineering construction from titanium nickelide and suspension of autologous blood mononuclear cells. Stained with hematoxylin and pikrofuksin by the method of Van-Giezon. ×300

(4386.3 ± 498.1 cells per 1 mm^2 section) and a large number of newly formed vessels were found around the titanium nickelide implant (Table).

In animals of group 2 (6 rats), on the 21st day after the operation, a loose connective tissue with thin collagen fibers disordered around the titanium nickelide implant was found in the cavity of the eyeball stump. Between bundles of collagen fibers, diffuse mononuclear infiltration (2020.6 ± 562.8 cells per 1 mm^2 section) and a small number of newly formed vessels were found (Table, Fig. 5).

In animals of group 3 (6 rats) on the 21st day after the operation, adipose tissue was detected in the cavity of the eyeball stump, insignificant mononuclear infiltration was observed between the lobules (106.1 ± 43.5 cells per 1 mm^2 section) together with loose connective tissue (Fig. 6). Around the Alloplant biomaterial implant, single moderately full-blooded vessels were found (Table).

According to morphometry, in the cell composition of the eyeball stump in rats of all 3 groups throughout the experiment (21 days), mononuclear leukocytes population prevailed. Moreover, in animals of the 1st group with an implant from a tissue-engineering construct, the cells number in this population during the experiment was statistically significantly higher than that in animals of the remaining groups.

Indeed, on the 7th day after the operation, the number of mononuclear leukocytes in the eyeball stump in rats of the group 1 was 3.3 times ($p_U = 0.034$) higher than in animals of group 2 with the titanium nickelide implant and 56.1 times ($p_U = 0.0002$) higher in animals of group 3 with the Alloplant biomaterial implant. On the 14th

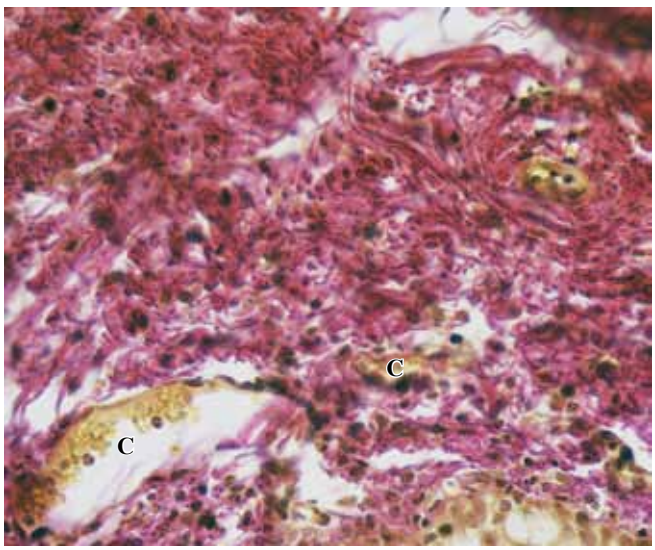

Fig. 5. Loosely arranged bundles of collagen fibers and moderately full-blooded vessels in the orbital stump of the 2nd group of animals on the 21st day after evisceroenucleation with implantation of titanium nickelide. C – vessels. Stained with hematoxylin and pikrofuksin by the method of Van-Giezon. ×400

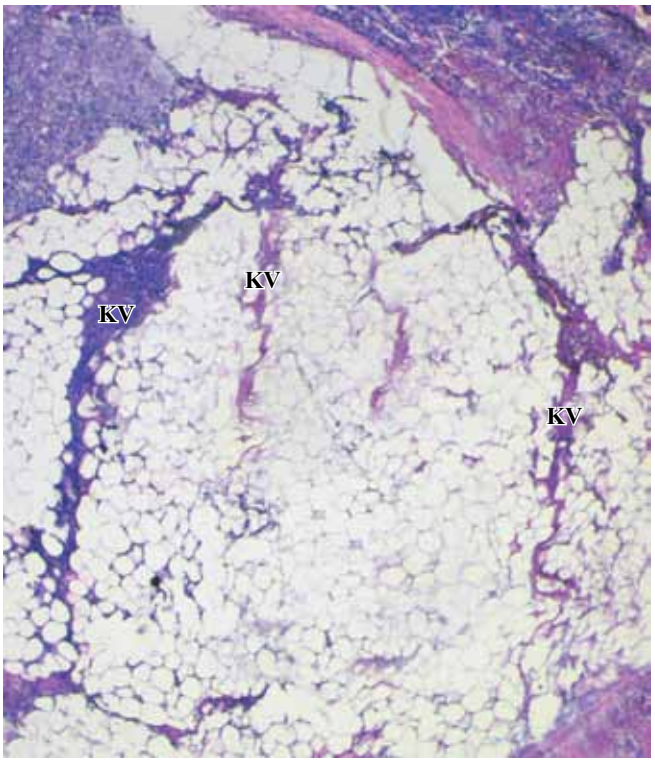

Fig. 6. Loose collagen fibers between the segments of adipose tissue in the orbital stump of the 3rd group of animals on the 21st day after evisceroenucleation with implantation of biomaterial "Alloplant". KV – collagen fibers. Stained with hematoxylin and eosin. ×200

day, the number of cells of this population in the eyeball stump in rats of the group 1 exceeded that in rats of group 2 by 1.9 times ($p_U = 0.01$), while in rats of group 3 – by 87.7 times ($p_U = 0.0001$), on the 21st day – 2.2 times ($p_U = 0.02$) and 41.3 times ($p_U = 0.0002$), respectively. The discovered regularity is explained both by the direct introduction of a suspension of autologous mononuclear leukocytes of blood into the tissue-engineering structure during the formation of the eyeball stump in group 1 rats and by the additional migration of cells of this population due to the inducing effect of exogenously introduced mononuclear cells.

The specific volume of connective tissue in the eyeball stump in animals of group 1 with the tissue-engineering implant, from the 7th day after the operation and throughout the experiment, has also been statistically significantly higher than that in animals of the other groups. On the 7th day the operation, this indicator in rats of group 1was 7.9 times ($p_U = 0.048$) higher than in animals of group 2 with a titanium nickelide implant and 15.8 times ($p_U = 0.039$) higher than in animals of group 3 with the Alloplant biomaterial implant (Table). On the 14th day after the operation, the volume of connective tissue in the eyeball stump in animals of group 1 reached the highest value compared to that in animals of other groups.

On the 21st day after the operation, in animals of group 1, morphometry revealed decrease in the stroma volume of the eyeball stump by 1.2 times ($p_U = 0.0019$) compared with the value on the 14th day (Table), which is due to the maturation of connective tissue in the eyeball stump. In animals of group 2, on the contrary, the stroma volume of the eyeball stump on the 21st day reached the highest value both in comparison with the initial data and in comparison with those in the rats of the other groups (Table). However, in the connective tissue of the eyeball stump, all animals of group 2 (6 rats) under light microscopy showed signs of immaturity. In animals of group 3, although the stroma volume of the eyeball stump on the 21st day after the operation exceeded that on the 14th day by 26.5 times; nevertheless, its level was of the least importance compared to rats of the other groups (Table).

The numerical density of newly formed vessels in the eyeball stump in animals of group 1, from the 14th day after the operation and till the end of the experiment (21st day), was statistically significantly higher than that in rats of the other groups (Table). Moreover, on the 21st day, in rats of group 1, this indicator was 4.0 times ($p_U = 0.001$) higher than in animals of group 2 and 9.8 times ($p_U = 0.0003$) higher than in animals of group 3 (Table).

DISCUSSION

Mononuclear leukocytes of blood, due to the synthesis and secretion of a large number of cytokines (interleukins – IL-1α, IL-1β, IL-6, IL-10, tumor necrosis factor, and vascular endothelial growth factor) are known to inspire the migration of mononuclear cells, PML and fibroblasts, accelerate proliferation of fibroblasts and endotheliocytes, affect the complement system and collagen production [14, 15, 16]. Probably, due to the functional cooperation of mononuclear leukocytes exogenously introduced into the structure of a titanium nickelide implant placed in the rat eyeball stump and additionally migrating cells under their influence which ensure the development of an inflammatory-reparative reaction, and the rapid change in cell phases occurs accelerating the transition of inflammation to the repair phase [7, 15]. At the same time, there is a rapid (within 21 days) maturation of connective tissue in the of the stump and accelerated neovasculogenesis starting at the 14th day.

It should be noted that the use of titanium nickelide as the basis of the implant, due to the porous structure of the material, significantly facilitates the germination of the implant with fibrovascular tissue. This, in turn, ensures its strong retention in the stump cavity of the eye of the experimental animal and significantly reduces the risk of the implant exposure and rejection [15, 16]. During the *in vivo* experiment with a titanium nickelide implant (36 rats, 36 eyes) no complications (infection, implant rejection) were revealed in the postoperative period. According to published data [1, 2, 4], the risk of

postoperative complications with orbital implants from various synthetic materials varies from 4 to 38%.

In addition, the titanium nickelide implant, due to the elastic properties of the material, provides a stable form of the eyeball stump.

CONCLUSION

Implantation of titanium nickelide tissue-engineered construct and a suspension of autologous blood mononuclear leukocytes into the scleral sac after evisceroenucleation in an *in vivo* experiment leads to accelerated maturation of the connective tissue and intensive vascularization in the eyeball orbital stump. This ensures strong fixation of the implant and reduces risk of rejection. The results are promising for further clinical studies.

The authors declare no conflict of interest.

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TISSUE EQUIVALENT TRANSPLANTATION IN THE TREATMENT OF CERTAIN SKIN INJURIES

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Chronic ulcers are a common and socially significant problem worldwide. Autodermoplasty is the gold standard treatment for chronic ulcers. However, it is not always possible to perform this surgical procedure for a rather large group of patients, due to some reasons, which include high risk of autodermotransplant rejection, lack of donor material, and patient's unwillingness to undergo surgery with an often unpredictable result. A potential solution to the problem is to use skin equivalents from allogeneic donor material. The use of allogeneic (donor) human cells makes it possible to fill the deficit of the patient's donor resources and close wound without causing additional injury to the patient. This paper provides an overview of the application of foreign and domestic biomedical cell products in clinical trials and real clinical practice. We draw conclusions on the efficiency of the considered biomedical cell products in the treatment of chronic ulcers, evaluate the conducted research, and make recommendations on the most efficient use of allogeneic dermatotropic biomedical cell products.

Keywords: biomedical cell products, dermatotropic products, bioengineered constructs, skin equivalents, burns, diabetic ulcers, diabetic foot, multipotent mesenchymal stromal cells, keratinocytes.

Despite the fact that surgery has been used to deal with superficial wound epithelialization, the problem is far from being solved. This claim is illustrated by the high prevalence of chronic wounds among patients admitted for purulent surgery [1].

The purpose of this review is to summarize the well-known practice of treating skin injury, mainly chronic wounds, using products containing human living cells, called biomedical cell products (BCPs) in the Russian Federation.

First of all, it should be noted that there are certain features in the definitions of terms and concepts related to skin injuries. The terms "trophic ulcer" and "long-lasting non-healing wound" are common in Russia. A trophic ulcer can be called a dermal defect caused by a disease and lasting longer than 3 months [2]. A long-lasting non-healing wound is any wound defect resulting from an injury (including surgical injury) with a low repair rate. As can be seen, the criteria are not precise, and separation of wound types in no way affects the volume and sequence of treatment and diagnostic measures.

Most foreign researchers in their practice use exclusively the "long-lasting non-healing wound" concept; this term refers to the following pathological processes: lower limb trophic ulcers, diabetic ulcers, pressure ulcers, non-healing wounds, wounds developing at the site of an injury or surgical intervention, as well as wounds resulting from a frostbite, heat, or chemical damage to the skin [3]. According to the U.S. Food and Drug Administration (FDA), any wound that does not heal within 30 days of standard care is chronic [4].

Generalization of these processes, different in terms of cause and pathogenesis, is quite justified if they are considered in terms of clinical manifestations and processes occurring at the cellular and subcellular levels [3]. Investigating morphological changes in biopsy materials taken from chronic wounds of various types, Fedorov et al. indicated that any chronic wound (regardless of cause) is associated with similar morphological and functional disorders [5]. First of all, the functioning of cellular elements in a wound becomes pathological, extracellular matrix synthesis and remodeling processes get impaired, formation of a basement membrane and migration of keratinocytes become impossible. These changes include predominance of monocytic-macrophagal cells in the inflammatory infiltrate in combination with insufficient number of T-helper/T-suppressor cells; decreased content of type III collagen, accumulation of tenascin and plasma fibronectin, a change in the typical localization of laminin, minimal number of myofibroblasts in the

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wound defect site; changes in the mediator systems regulating the course of inflammatory-reparative response: increased accumulation of pro-inflammatory cytokines (IL-1β, MIP-lα/MIP-1β) and disruption in their normal ratio, absence of fibrogenic growth factors (TGF-β1, bFGF) in combination with increased activity of matrix metalloproteinase – 9 in the wound area [2]. There are always signs of chronic inflammation. Also, a chronic wound is characterized by low angiogenic tissue response [6], impeding neovascularization and healing.

Thus, a long-lasting non-healing or chronic wound is a dermal defect resulting from external exposure (mechanical, heat, chemical damage) or surgical intervention, existing for more than 30 days. It has a low self-healing tendency and characterized by the presence of certain changes that impede repair, creating self-sustaining system, operating like a "vicious circle". Detection of these histological and immunohistochemical signs allows to verify a chronic wound, but there are currently no clear morphological criteria for diagnosing chronic wounds.

Diabetic neurotrophic foot ulcer is one of the most typical chronic wounds [7, 8]. The non-healing nature of diabetic ulcer is explained by both abnormal inflammation phase and wound cleansing, and abnormal proliferative phase [9].

It is logical that transplantation of full-fledged skin or its elements – the intercellular matrix (hereinafter referred to as the scaffold), skin cells and dermis – can restore disrupted proliferative processes.

Autodermoplasty is the gold standard for treating chronic wounds. However, its implementation is not always possible in a sufficiently large group of patients. One of the reasons is the high risk of rejection of the autodermal graft from the wound surface, shortage of donor material, and the patient's unwillingness to undergo surgery with an often-unpredictable result. The outcomes are especially unsatisfactory in patients with neuropathic wounds amidst diabetic foot syndrome.

Various collagen dressings have been suggested as an alternative to own skin. However, they are significantly inferior in terms of effectiveness to allogeneic (taken from a donor) cell dressing material, and even more so when transplanting the skin [4]. However, collagen dressings can be used as a means of preparing for transplantation of cell structures or as a basis for creating various three-dimensional structures. Scaffolds of tissue-engineered skin substitutes are located inside the wound defect and play the role of a biological dressing, providing protection against dehydration, microorganism invasion, and toxin penetration. It is then embedded in the wound bed through natural wound healing mechanisms, such as inflammation, cellular infiltration by neutrophils, macrophages and fibroblasts, and the scaffold is neovascularized. Biocompatibility of the carrier matrix can be enhanced by adding to its structure fibroblasts that can accelerate skin regeneration processes. Dermal fibroblasts produce all the main components of the intercellular matrix (collagen, glycosaminoglycans, proteoglycans) and are also responsible for continuous matrix remodeling process. Fibroblasts are an active cellular component that can structure the dermal collagen, stimulate the growth of granulation tissue in the wound and secrete a number of growth factors promoting development of a neovascular network, formation of basement membrane and cellular migration, which accelerates skin regeneration. However, this composition leads to wound defect filling without epithelial regeneration.

Such reflections led to the creation of a heterogeneous group of products that are designed to provide complete wound closure by reconstructing the skin defect and taking over the function of the missing skin layer. An ideal skin equivalent would accelerate neoangiogenesis, extracellular matrix remodeling, granulation tissue formation and skin regeneration. The skin equivalent should lead to formation of new blood vessels and maturation of the neovascular network, resulting in decreased inflammatory process, effective healing with less scarring and wound contraction.

In the Russian Federation, some allogeneic skin equivalents are currently developed, studied under laboratory conditions, and until recently, were used in clinical practice, tested for treatment of patients with chronic non-healing wounds:

Human living skin equivalent (LSE) [10]. Ivashkin A.N. (2009) studied an LSE for the treatment of chronic wounds. The LSE is a gel-like substance composed of a synthetic mesh base. Fibroblasts are distributed in its three-dimensional structure; the surface is covered with keratinocytes [3]. LSE was used to treat patients with skin wound defects that did not heal for at least 1 month of adequate care. The patients included 22 people in the main group, and 20 in the control group. Patient groups were comparable in age, severity of accompanying conditions, initial wound defect area (29.5 ± 3.5 cm in the main group and 31.1 ± 3.6 cm in the control group). Differences in wound defect sizes did not exceed 15% within a group. The rate of decrease in the wound surface area in the main group was two times higher than in the control. Most patients with long-lasting non-healing wounds (in 81.8% of cases) underwent a single LSE plastic surgery. By the end of week 4 in the main group, healing had begun in most patients (16.7%) or there was significant decrease in wound size (41.7%). After day 25 in the control group, wound sizes decreased by less than 40%. In 20% of patients, complete epithelization did not occur at a later date. No histological and immunohistochemical verification of chronic wounds was performed before the patients were included in the study. Histological examination of wound biopsy was performed on day 5, 10, 20 of the study after treatment had started in the main and control groups. Histological signs of the onset of wound contraction process, and

granulation tissue maturation were noted in the main group on day 5. On day 10, mature granulation tissue was detected, which resembled the structure of a normal dermis, while inflammatory infiltration persisted. On day 20, biopsies of the main group showed signs of active wound reepithelization. The structure of the underlying tissue resembled a normal dermis. Inflammatory infiltration was minimal in most cases. In the biopsy specimens of the control group, such pronounced signs of healing were not observed, and inflammatory phenomena persisted for a long time. Thus, despite lack of a description of the study methodology and the small sample size, we can conclude that LSE has a significant clinical efficacy. It was studied in comparable groups with equivalent wound defect sizes, and the observed wound surface area reduction in the main group, in comparison with the control group, was confirmed by histological examination results, where the predominance of reparative processes in the LSE treatment group was clearly traced.

Dermal equivalent (two modifications: in collagen gel and based on plasma fibrin or fibrinogen). The dermal equivalent contains allogeneic dermal fibroblasts encapsulated in collagen gel. In a study by Kotslova et al. which evaluated the efficacy and safety of dermal equivalent in the treatment of wounds in patients with diabetic foot syndrome, 60 patients with granulating wounds, lasting more than 6 weeks, which did not reduce by 50% or more after 2 weeks of standard care, were included [11, 12]. The main group consisted of 40 patients; the control group had 20. All patients had superficial ulcers with no signs of infection; some patients had signs of ischemia (1A, 1C according to the University of Texas Wound Classification System). Within the groups, there was significant variation in wound defect sizes: from 1 to 25cm^2. Histological verification of chronic wounds was not performed, no histological examination of biopsy samples of wounds in the course of treatment in the groups was reported in publications. Researchers noted that ischemia significantly reduced epithelialization rate, smaller wounds healed faster, wounds more than 12 cm^2 required repeated application of the dermal equivalent within 3 to 4 months from the start of treatment. Healing was not achieved within 1.5 years in 25% of patients in the main group. This was mainly due to the lack of healing in patients with ischemia. The average complete healing timeframe in the main group ranged from 1 to 3 months (an average of 67 ± 11 days); complete healing was achieved in 65% of patients in the main group. However, it is not indicated which percentage of patients had complete wound healing 1 month after application of the dermal equivalent. The study was limited by the small sample size; the studied groups were heterogeneous in terms of wound defect sizes and number of patients; there was no description of the chosen research methodology and methods for assessing and comparing indicators in such heterogeneous groups. The average epithelialization rate in the groups after 1 and 2 months is given. The healing dynamics in the groups is not given. Thus, based on the published study, it is difficult to firmly conclude on the true effect of the dermal equivalent on wound healing.

According to Varkey et al, the following dermatotropic BCPs containing human cells were registered as of 2015 (Table 1) [14].

Table 1

List of registered dermatotropic BCPs as of 2015

Product	Composition	Comments
TransCyte®	Nylon mesh seeded with neonatal human foreskin fibroblasts that are destroyed before grafting	Temporary wound dressing upon which autografts are placed
Dermagraft®	Bioabsorbable polygalactin mesh matrix seeded with human neonatal fibroblasts and cryopreserved	Matrix facilitates re-epithelialization by the patient's own keratinocytes
Apligraf®	Bovine collagen gel seeded with neonatal foreskin fibroblasts and keratinocytes	Wound dressing with two different cell types
OrCel®	Type I collagen matrix seeded with neonatal foreskin fibroblasts and keratinocytes	Wound dressing with two different cell types
Epicel®	Sheets of autologous keratinocytes attached to petrolatum gauze support	Wound dressing with autologous cells
StrataGraft®	Full thickness skin substitute with dermal and fully differentiated epidermal layers	Made with naturally immortalized NIKS® keratinocyte cell line; contains two different cell types
Tiscover® (A-skin)	Autologous full thickness cultured skin for healing of chronic, therapy resistant wounds	Contains two different cell types
Permaderm®	Autologous tissue engineered skin consisting of epidermal and dermal cells	Contains two different cell types
denovoDerm™	Autologous dermal substitute	To be used in combination with split-thickness skin grafts
denovoSkin™	Autologous full thickness substitute consisting of dermal and epidermal layers	Contains two different cell types

It should be noted that out of 16 products containing human cells listed in these two reviews, 9 products (medical devices) are autologous, and 4 products are manufactured using cell material obtained from newborns. According to Russian law, minors cannot be cell donors in the production of BCPs, except for manufacture of autologous products.

The following skin substitutes are registered for treatment of chronic wounds: Apligraf®, Dermagraft®, TransCyte®, and OrCel®. StrataGraft® is in Phase III of clinical trials and is designed to treat severe deep burns. An FDA approval decision is expected in 2020. One cell type – allogeneic fibroblasts – contains TransCyte® and Dermagraft®. BCPs containing allogeneic keratinocytes and fibroblasts include Apligraf®, OrCel® products, which are widely and commercially available, as well as StrataGraft®, which is undergoing clinical trials.

The results of some clinical trials of BCPs are presented below.

Apligraf® (Organogenesis, USA). In a multicenter, randomized clinical trial (RCT) involving 72 patients, the outcome of using Apligraf® with standard therapy and the outcome of suing only standard therapy for the treatment of diabetic ulcers were compared. By 12 weeks, there was significant decrease in time to complete wound closure in the main group (51.5%) compared with the control (26.3%) [15]. In another multicenter, RCT involving 208 patients, who were randomly assigned to ulcer treatment either with Graftskin® (formerly Apligraf®) or saline-moistened gauze, 56% Graftskin-treated patients achieved complete wound healing compared with 38% in the control group at the 12-week follow-up visit. The Kaplan-Meier median time to complete healing was also significantly lower for Graftskin (65 days) than the 90 days observed in the control group. Osteomyelitis and lower-limb amputations were much less frequent in the experimental group [16].

Dermagraft® (Shire Regenerative Medicine, Inc., USA). A multicenter, RCT was carried out in 314 patients with chronic diabetic ulcers using Dermagraft® (main group) or conventional therapy (control group). By 12 weeks, 30.0% of Dermagraft patients had their wounds completely closed compared with 18.3% in the control group. Although the overall incidence of adverse events was similar for both groups, the number of patients who developed ulcer–related adverse events (infection, osteomyelitis, and cellulitis) was 19.0% in the Dermagraft-treated patients compared with 32.5% in the control patients [17]. A clinical study in 28 patients with chronic diabetic ulcers (longer than 6 weeks) compared Dermagraft® intervention with the control group (saline-moistened gauze alone). By week 12, 71.4% of ulcers healed in the Dermagraft group and 14.3% in the control group. Healed Dermagraft patients achieved wound closure significantly faster than the control group patients [18].

In a randomized, single-blind, clinical trial DOLCE comparing the differences between cellular-free, cellular (Dermagraft®) matrices and standard treatment for diabetic ulcers, skin substitutes showed an advantage [19]. In a Dermagraft® multicenter clinical trial, 62 patients were dressed with wet gauze or polyurethane foam bandages weekly after surgical treatment for ulcers. About 44% of patients had complete wound closure by week 12, and 52% healed by week 20. Median time to healing was 13 weeks. Dermagraft® has been shown to be safe and effective in the treatment of non-healing diabetic ulcers [20]. A multicenter RCT was performed to evaluate wound healing in 50 patients with diabetic foot ulcers. These patients were randomized into four groups (three different dosage regimens of Dermagraft® and one control group). Ulcers treated with the highest dosage of Dermagraft® healed significantly more often than those treated with conventional methods; 50% of the Dermagraft-treated and 8% of the control group healed completely [21].

TransCyte® (Shire Regenerative Medicine, Inc., USA). A clinical study using TransCyte® and silver sulfadiazine was performed with the use of paired wound sites on 14 patients. Wounds treated with TransCyte® healed much faster to a re-epithelialization state (mean 11.14 versus 18.4 days). Wound evaluations showed that at 3, 6 and 12 months, wound sites treated with TransCyte® healed with significantly less hypertrophic scarring than sites treated with silver sulfadiazine [22].

OrCel® (Forticell Bioscience, USA). To study a product for the treatment of chronic wounds, studies were conducted on patients with unhealed venous and diabetic ulcers. Clinical trials evaluating the efficacy of OrCel® for treatment of venous leg ulcers showed that 50% of OrCel® patients achieved complete wound closure at week 12 compared with 31% of subjects who received only standard therapy. Patients who received OrCel® exhibited a median time to heal of 77 days, whereas no median time was determined for the control group, since many ulcers did not epithelize completely. Results from the OrCel® pilot study in the treatment of diabetic foot ulcers show that 47% of patients in the experimental group achieved complete wound closure by week 12 compared to 23% of patients who received only standard therapy. In November 1999, OrCel® took part in a pilot study for 40 patients with diabetic foot ulcers using an updated version of the product. According to the data presented for 16 patients, it was found that at week 12, 56% of patients receiving OrCel® achieved complete wound closure, compared with 29% of patients receiving conventional care [23].

OrCel® is similar to Apligraf® because it contains both fibroblasts and keratinocytes derived from the foreskin of newborns, but uses a collagen sponge with type I collagen as matrix [24]. It is used to compensate for negative tissue defects in the wound, where it acts as a matrix for migration of the patient's own cells. In a study

that directly compared OrCel® with BiobraneTM for treating partial thickness donor wounds, OrCel®-treated areas had higher healing rates and reduced scarring. This healing improvement is due to the presence of a collagen sponge in combination with cytokines and growth factors produced by viable allogeneic cells [11].

It should be noted that when selecting clinical trials to assess their quality, the FDA chose a very limited number of trials: 95 were examined, 18 were selected [4]. Among the products considered for treatment of chronic wounds, Apligraf® and Dermagraft® studies were selected. Thier methodological quality significantly distinguished the studies of these products from the rest. Apligraf® study methodology was noted to be better than that of Dermagraft®. According to a Cochrane systematic review, Apligraf® showed a statistically significant positive effect on complete ulcer closure [25]. These data suggest that allogeneic cell products Apligraf® and Dermagraft® have most convincingly proven their effect on the healing of chronic wounds.

For the treatment of chronic wounds, Nathoo et al. recommend composite allogeneic skin substitutes in the treatment of wounds lasting more than 4–6 weeks, cell-free allogeneic skin substitutes, dermis substitutes, xenografts for other chronic wounds [26]. However, the use of bioengineered skin substitutes, according to Garwood et al., may depend on the ability of the substitute to synthesize the components of the dermis [27]. The authors distinguish dermoinductive (Apligraf®, Dermagraft®, etc.) and dermoconductive (dermo-substituting) products (Integra™ and others). From the authors' point of view, the choice of product should depend on the wound depth. For superficial wounds ending at the subcutaneous tissue, a dermo-inductive product is recommended. For injuries reaching the subcutaneous tissue and deep tissue, a dermoconductive product should be considered; if there is no neodermis formation, autodermoplasty should be contemplated.

Despite the fact that Law No. 180 FZ on Biomedical Cellular Products came into force in January 2017, until now dermatotropic BCPs were not produced on a commercial scale in the Russian Federation, and so far, there are no products approved for clinical use.

Given the number of BCPs in the world that have been gradually approved and, in some cases, withdrawn in recent years, the International Society for Cell and Gene Therapy (ISCT) has presented a brief annual report on cell products approved for clinical use in different countries [13]. The authors report that this list may not be exhaustive and that to the best of their knowledge, no cell/tissue/gene products have been authorized for marketing in Brazil, Hong Kong, Israel, Malaysia, Singapore and Taiwan as of September 2018. So, according to ISCT, as of September 2018, 6 dermatotropic products satisfying the criteria of BCPs in Russia are allowed for clinical use in the world (see table 2).

It should be noted that many products from the 2015 list are missing from the new list, and StrataGraft® BCPs are fundamentally different in composition from the 2015 version. All this indicates that the global market for BCPs, including dermatotropic ones, is still forming.

CONCLUSIONS

A study of the use of various skin equivalents suggests that BCPs have advantages in the treatment of chronic wounds. It was found that in chronic wounds, the patient's own cellular and extracellular elements are pathologically altered, and their physiological functions are impaired. In chronic wounds, the intensity of repair processes is reduced, and this therefore necessitates introduction of cellular elements from the outside, while

Table 2

List of dermatotropic BCPs approved for clinical use as of 2018

Name	Composition	Comments
JACE® (J-TEC)	Autologous cultured epidermis	For the treatment of severe burns (Japan); in the market since 2007
KeraHeal-Allo™ (KeraSkin, Biosolution Co., Ltd.)	Composite cell product – spray (allogeneic skin-derived keratinocytes suspended in a thermosensitive hydrogel)	For deep 2nd degree burns (South Korea); in the market since 2015
Kaloderm® (Tego Science, Inc)	Allogeneic keratinocytes (cell sheet)	For deep 2nd degree burn (in the market since 2005) or diabetic foot ulcer (South Korea) (in the market since 2010)
KeraHeal® (Biosolution Co., Ltd.)	Autologous keratinocytes	For deep 2nd degree burns that cover >30% of the total body surface area (TBSA) and 3rd degree burns that cover >10% of TBSA (South Korea); in the market since 2006
Holoderm® (Tego Science, Inc)	Autologous keratinocytes	For deep 2nd degree burns that cover >30% of TBSA and 3rd degree burns that cover >10% of TBSA (South Korea); in the market since 2002
StrataGraft® (Mallinckrodt plc).	Autologous skin cell product	For the treatment of deep partial thickness burns (USA); in the market since 2017

replacing the tissue mass deficit in the case of deep, full-thickness wounds.

Own skin grafting is not a reliable treatment method. It often turns out to be unsuccessful, which is unacceptable for a number of situations. Most of these patients require cellular and non-cellular elements from the outside as part of the cellular product for successful proliferation. At the same time, fibroblasts or mesenchymal stem cells should be an indispensable component of BCPs as a central element of the repair process, promoting neovasculogenesis, extracellular matrix remodeling, basement membrane synthesis, and keratinocyte migration. In patients with type 2 diabetes mellitus, keratinocyte-containing products should be used, since chronic hyperglycemia changes the morphology of cells, reduces cell proliferation and inhibits keratinocyte differentiation.

Available publications suggest that the use of dermatotropic BCPs with biodegradable collagen structures is promising. However, lack of comparative clinical studies and a single protocol can sometimes significantly reduce the importance of individual clinical observations.

It should be noted that when using dermatotropic BCPs, the wound itself must be adequately prepared. Preparation for application is a requirement for all BCPs before use to ensure the best possible outcome. Complex treatment, wound cleansing, including surgical treatment, reduction of infectious load, relief of the affected limb, daily care with assessment of the wound process dynamics can create prerequisites for successful use of BCPs in the treatment of chronic wounds.

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GLOBAL ORGAN SHORTAGE: AN ANALYSIS OF NATIONAL SELF-SUFFICIENCY STRATEGIES

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From the standpoint of socio-humanitarian knowledge, the paper analyzes the problem of global organ shortage. The basic ideas of the international medical community about organ shortage and the main proposals for overcoming it are considered. Special emphasis is placed on the three most revealing national self-sufficiency strategies adopted by donor agencies – American, Spanish and Iranian strategies. The issue of influence of cultural differences and socio-economic inequality on established organ donation practices is discussed using Mexico, Turkey, Pakistan and Bangladesh as examples.

Keywords: organ shortage, WHO, national self-sufficiency strategies, USA, Spain, Iran, Mexico, Turkey, Pakistan, Bangladesh.

INTRODUCTION

Advances in transplant medicine as a result of better surgical techniques, postoperative rehabilitation and use of effective immunosuppressive drugs have made organ transplantation a routine medical practice in clinical settings around the globe. As a result, since the 1990s, organ donor shortage has been the main problem preventing efficient provision of transplant medical care for patients in need of it. This phenomenon is not specific to a particular country but a global challenge. Global organ shortage requires special study, as does organ shortage within a specific national jurisdiction.

The following issues are to be discussed under this paper:

– What is organ shortage from the perspective of the international medical community?
– What organ shortage management strategies are currently being proposed?
– How are cultural differences and socioeconomic inequalities affecting organ self-sufficiency practices?

WHO ON ORGAN SHORTAGE

From the moment organ transplantation became a successful means of saving lives, the international medical community, represented by the World Health Organization (WHO), began to pay a closer attention to various issues related to transplantation practices and donation. In 1987, the 40th World Health Assembly decided to develop the "Guiding Principles for Human Organ Transplants", which would highlight the progress achieved in human organ transplants and affirm that trade for profit in human organs among living human beings is inconsistent with the most basic human values and contravenes the Universal Declaration of Human Rights and the spirit of the WHO Constitution [1].

In 1991, WHO adopted the "Guidelines on Human Organ Transplantation" [2], which noted a shortage of donor organs and stated that "supply has never satisfied demand". Major focus was placed on the issue of organ trafficking from unrelated donors and special concern was also expressed on the fate of various vulnerable groups who became victims of trafficking. In order to stop this trade, the following principles were put forward: (1) organs should preferably be obtained from the deceased, (2) living donors should generally be genetically related to recipients, (3) no payment should be given or received for organs [3].

Subsequently, WHO repeatedly returned to the issue of organ shortage, invariably linking it with the problems of commercial organ trafficking, which was considered a serious obstacle to the normal development of transplantation worldwide. So, in 2004, the 57th World Health Assembly, taking into account already gained experience and new trends in transplantation practice, recommended revising the 1991 "Guidelines". Special emphasis was placed not only on organ trafficking, but also on transplant tourism. Speaking of "the growing insufficiency of available human material for transplantation", WHO also recognized that "transplantation encompasses not only medical but also legal and ethical aspects, and involves economic and psychological issues" [4].

In March 2007, the second Global Consultation on Transplantation took place in Geneva. During the event,

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WHO presented the stakeholders with a blueprint for updating the Guidelines. The stakeholders agreed to the creation of a Global Forum on Transplantation to be spearheaded by WHO, to assist and support developing countries initiating transplantation programs and to work towards a unified global coding system for cells, tissues and organs. During the Geneva consultation, it was noted that in 2005, 66,000 kidneys were transplanted in the world, but this represented a mere 10% of the estimated need. In addition, it was reported that "transplant tourism" makes up an estimated 10% of global transplantation practices. WHO experts emphasized that "quality, safety, efficacy and transparency" are essential if society is to reap the benefits transplantation can offer as a therapy" [5].

Many of the ideas voiced during the Global Consultation in 2007 were presented as part of the 2008 Declaration of Istanbul on organ trafficking and transplant tourism. Several important approaches to the issue under consideration were expressed in the declaration. "All countries need a legal and professional framework to govern organ donation and transplantation activities, as well as a transparent regulatory oversight system that ensures donor and recipient safety and the enforcement of standards and prohibitions on unethical practices." "Each country should strive both to ensure that programs to prevent organ failure are implemented and to provide organs to meet the transplant needs of its residents from donors within its own population or through regional cooperation." The authors of the declaration called on all participants in international communication to expand the "therapeutic potential of deceased organ donation," "minimize the burden on living donors," eliminate "barriers, misconceptions, and mistrust that currently impede the development of sufficient deceased donor transplantation," and improve the health infrastructure. Within the framework of the declaration, six principles were formulated, one of which stated: "Jurisdictions, countries, and regions should strive to achieve self-sufficiency in organ donation by providing a sufficient number of organs for residents in need from within the country or through regional cooperation" [6].

In 2010, the 63rd World Health Assembly approved the new "Guidelines", in which the "Declaration of Istanbul" idea was implemented. Particular attention was again paid to the challenges of transplant tourism and organ trafficking, which was closely associated with human trafficking. Eleven principles proposed to the world community emphasized the following: any consent required by law should be obtained before cells, tissues or organs (CTOs) may be removed from the bodies of deceased persons for the purpose of transplantation; physicians determining the death of a potential donor should be different from those directly involved in CTOs removal from the donor or subsequent transplantation procedures; donation from deceased persons should be prioritized over donation from living donors, and living donors should be genetically, legally or emotionally related to their recipients; no CTOs should be removed from the body of a living minor for the purpose of transplantation other than narrow exceptions allowed under national law; CTOs should only be donated freely, without any monetary payment or other reward of monetary value; high-quality, safe and efficacious procedures are essential for donors and recipients alike; the organization and execution of donation and transplantation activities, as well as their clinical results, must be transparent and open to scrutiny, while ensuring that the personal anonymity and privacy of donors and recipients are always protected [7].

In view of the Declaration of Istanbul, and the 63rd World Health Assembly Resolution, leading WHO experts soon published a special text urging governments of all countries to seek tighter control in achieving self-sufficiency in organ donation and transplantation. The authors of the publication specifically emphasized that "a new paradigm of national self-sufficiency is needed" and reiterated that "each country or region should strive to provide a sufficient number of organs from within its own population, guided by WHO ethics principles." The published material also contained a variety of information about the situation with the self-sufficiency of donor agencies in various countries of the world [8].

In general, during the late 1980s and early 2010s, WHO always adhered to a line of behavior that was aimed at addressing the problem of global organ shortage. In the course of this, it gradually came to the realization that many phenomena were hindering the fight against organ shortage – inconsistencies in national legislation on donation and transplantation, dishonesty among some members of the global transplant community, organ trading and transplant tourism. Almost from the very beginning, it was also recognized that there were major differences between developed and developing countries on how to organize effective and fair donation systems. Contradictions regarding the acceptability of the use of deceased or living donors and their attitude to organ sale were also revealed. Recognizing these differences and contradictions, leading WHO experts, however, are increasingly asserting that overcoming organ shortages should be a common goal for all participants in international communication, and the means for this should be on working towards self-sufficiency in donor organs within each country or region.

NATIONAL SELF-SUFFICIENCY STRATEGIES

Unavoidable organ shortage not only remains a constant headache for the international medical community, but also a starting point for developing various strategies to address it. The national organ self-sufficiency concept proposed by WHO is a framework, but each country

or group of countries may have its own approaches to practical implementation. Below we will consider some of the best-known self-sufficiency strategies for organ donation.

U.S. strategy

Along with the Soviet Union, the United States was one of the pioneers in the field of organ transplantation. One of the world's first successful organ donation systems was created there, which made it possible to harvest organs on a national scale and quickly redistribute them among medical institutions. In 1968, first organization, professionally engaged in organ donation – the New England Organ Bank (NEOB) – was established. It employed special specialists involved in identifying donors in hospitals located in the region, managing them after brain death diagnosis, obtaining consent to organ harvesting from the donor's relatives and providing psychological support to theь, receiving and transporting organs, monitoring the quality of work performed, organizing public information campaigns, media contacts, etc. Following NEOB, 57 more organ procurement organizations (OPOs) appeared [9, 10].

An important step was taken in 1984 when the US Congress approved the National Organ Transplant Act, after which the U.S. Department of Health and Human Services established the Task Force on Organ Procurement and Transplantation, comprising of specially appointed group of 25 specialists, to streamline the work of all regional OPOs. This led to the establishment of the national organ-sharing and procurement system through the Organ Procurement and Transplantation Network (OPTN), all of whose links were connected into a single computerized network. From 1986, this network and the entire OPTN started to be administered by non-profit, scientific and educational organization – the United Network for Organ Sharing (UNOS), overseen by the U.S. Department of Health and Human Services. The institutional members of UNOS include all US transplant centers and OPOs, managed through the Board of Directors and ad hoc committees [11, 12].

Along with national organizations OPTN and UNOS in the United States, regional associations started emerging in those same years, which included transplant centers, organ harvesting centers, local businesses and state medical administrations. They were focused on receiving and distributing organs to a higher level, to achieve material interest from the medical institutions involved in it, and also to optimize selection of candidates for organs, especially such scarce organs as transrenal organs. The best known of these regional associations was the Ohio Solid Organ Transplantation Consortium (OSOTC) created in Ohio in 1984 [13].

Due to the increasing number of medical solutions for transplantation treatment and the increasing demand for donor organs, the US national organ donation system became overloaded with work as early as the early 1990s, and this required subsequent adaptation to new challenges. Having started the struggle to increase the sources of donor organs, US specialists along with brain-dead donors began to more actively use "donors with advanced criteria", "marginal donors", "donors with heart failure", and also began to more widely attract various categories of intravital donors. In the 2000s, the professional language of US transplant doctors included such concepts as "living unrelated donors", "living donors legally and emotionally related to recipients", "directed and nondirected living donors", "good Samaritan donors" [14, 15].

The desire to use the full range of opportunities for obtaining new organs makes the U.S. self-sufficiency strategy one of the most aggressive in the world. It is noteworthy that the term "aggressive organ harvesting", which began to be promoted in scientific literature in the mid-2000s, is intended to refer to the tactics of "aggressive manipulation (management) of the donor's body in order to obtain maximum number of organs for transplantation". [16]. However, this notion seems to be true not only for characterizing specific medical situations, but also for the general trend in the US transplant medicine: to make the most of existing opportunities and create new ones.

Spanish strategy

Since 1990, Spain has had the most successful experience in organ self-sufficiency, with the establishment of the National Transplant Organization (*Organización Nacional de Trasplantes*) in 1989 under the leadership of nephrologist Rafael Matesanz, who led all organ donation activities based on a new model for obtaining donor organs – transplant coordination. For the first time in the world, Spain established a practice where identification and management of potential donors was entrusted on special specialists (transplant coordinators), whose activities were strictly accountable and paid for. Appointed from among hospital physicians, primarily from the intensive care units (ICUs), the transplant coordinators were able to ensure effective interaction between attending physicians and transplant teams, whereas in previous decades this was not possible. The whole chain of transplant coordination, from hospital to regional and national level, was built, and all technical and logistical aspects of quick access to donors and donor bodies were well-thought-out. While recognizing the importance of living donation, Spanish experts nevertheless focused on improving deceased organ donation. Within a few years the country came out on top in the world by these indicators. Consequently, WHO recognized the experience of Rafael Matesanz and his colleagues as exemplary and recommended it to other countries.

In addition to working to improve the situation with organ donation in their country, Spanish experts have also been actively involved in international expert analysis and advocacy on organ donation issues. With the participation of the Spanish National Organization, various pan-European and international documents on organ donation, such as guidelines, statistical information, declarations and directives, have been published. Thanks to persistent efforts by R. Matesanz, an article, for the first time in the world, was introduced into Spanish criminal law prohibiting trafficking in organs and severely punishing those who buy organs abroad. Calling transplant tourism a criminal and immoral activity, R. Matesanz pointed out that the activities are facilitated by doctors themselves, primarily in developed countries – the United States, Japan, Israel and Europe, supporting their patients who go in search of organs [17].

Spain's national self-sufficiency strategy, which actively promotes the idea of deceased organ donation to the public through the media, church and educational institutions, is nevertheless inferior to the U.S. strategy in terms of aggressiveness. For example, Spanish transplant doctors are almost twice less likely than their US counterparts to use the living donation potential and almost never use the living unrelated donation potential (in 2017 only 14 kidney transplants from unrelated donors were performed, which amounted to 0.3 cases per million people, while in the United States, 1124 such operations were performed, or 3.5 cases per million) [18]. Nevertheless, the idea of "aggressive organ harvesting" also finds support among Spanish specialists. An example of this is the proposal by Diego Gracia, Spain's most respected medical philosopher and bioethicist, to move from voluntary altruistic donation to compulsory civil obligation to transfer their organs after death. In his opinion, "organs of dead people are public goods", and therefore they should be disposed of not by an autonomous individual, but by the so-called "super-user" (supererogatory) – the whole society or the state. D. Gracia calls this approach a "radical solution" to the problem of organ shortage, but stresses that there are no legal grounds for this yet [19].

Iranian strategy

Another self-sufficiency strategy is related to the legalization of payment for organ donations. Officially, it is implemented only in one country, Iran, where it was introduced in 1988 immediately after the end of the Iran-Iraq war and under international political isolation. The role of the authoritarian theocratic regime in adopting this model of donor system in this Islamic state is not quite clear, but it is possible that the choice to legalize organ trade was a kind of reaction to the values of Western liberal democracy rejected by Iran and everything associated with it. Be that as it may, legalization of the sale of organs played a crucial role in the development of the national transplant program. The state became the buyer of all donor organs in Iran, thereby eliminating a number of moral, ethical and legal issues. The majority of Iranian specialists see this model as absolutely valid and fair [20, 21], although neither WHO nor most Western experts consider it as such.

The adoption of the paid model for acquisition of donor organs (kidneys) allowed Iran to get rid of long queues on waiting lists in just ten years. Thanks to this, the country was also able to put an end to illegal organ trafficking. With the introduction of a fee-based system, renal donors began to receive $3,500 for a sold organ (in the late 1990s), although the price later declined – $1,265 in 2002 and $900 in 2011. Besides, the Government provided free health insurance to donors. By supporting organ trade, the Iranian State had achieved a significant reduction in funds for high-tech medical care: the cost of maintaining patients at dialysis centers had been reduced. Both Western and Iranian specialists pay attention to the reasons why Iranians sell their organs. It is believed that these reasons are mixed – both financial and altruistic. However, organ sales are not just a matter for poor people. According to various estimates, the percentage of completely illiterate people selling their organs ranges from 2.7% to 29%, while the number of people with school education (6–12 years) ranges from 71% to 90.8%. In 2000, Iran passed a law allowing the use of organs from brain-dead patients. By the early 2010s, the number of kidneys received from such donors was 12%. Nevertheless, living unrelated donors continued to be the main source of organs in Iran in the early 2010s [22–25].

When comparing Iran's self-sufficiency strategy with that of the United States and Spain, it is easy to see that it clearly focuses on harnessing the potential of living unrelated donors. However, we have no reason to talk about the ideology of "aggressive organ harvesting" that is embedded in it. Unlike their US and Spanish counterparts, Iranian transplant doctors obviously do not take advantage of the full range of existing living donor opportunities. It seems that their choice is predetermined by the prevailing cultural norms of Islamic society, where a majority of the population is wary of the practice of removal of organs from deceased persons.

The three examples above do not exhaust the diversity of national self-sufficiency strategies that exist today, but they can be considered very indicative. Due to the role played in the world by US transplant medicine, the US "aggressive organ harvesting" strategy is in many ways a model for other countries, although it is not recognized as such by WHO. The desire to use the full range of existing opportunities to attract donor resources is the norm guiding the medical community in most developed countries. Spain's self-sufficiency strategy, recognized by WHO as an exemplary model, also has many supporters, apparently because it is less aggressi-

ve. The Iranian strategy, on the other hand, is officially unparalleled outside the country. However, sustained interest in it and abundance of publications on the role of the Iranian experience seems to have prepared this strategy for the future.

CULTURAL DIVERSITY AND SOCIAL INEQUALITIES

The international medical community, via WHO, attributes the possibility of developing transplantations and reducing the shortage of donor organs mostly to increased deceased organ donation. However, WHO experts rightly point out that in some parts of the world, the very idea of deceased donation triggers "cultural resistance". The countries in question are primarily those in Asia, Latin America and Africa, i.e. mainly developing countries. Recognizing this, WHO insists on the importance of fostering "qualitative research to understand the 'non-medical' reasons for this reluctance" [26, 27].

Mexico

Among developing countries in Latin America, Mexico is the most obvious example of the impact of culture on donor practices. This Catholic-dominated country is also governed by a government that has tried for decades to implement a modernization policy but is hopelessly lagging behind its northern neighbor, the US. Transplantation medicine is rather highly developed in the country. In 2017, Mexico was ranked fourth among all Latin American countries in terms of total number of kidney transplantations – 24.5 transplantations per million population. Meanwhile, the most frequent transplants are from living donors. Cadaveric donation is very underdeveloped. Mexico is one of the last countries in the region in terms of deceased organ use, but the number of kidney transplants with the use of living donors is 17.3. The total number of kidney transplants from deceased donors is more than twice less – 7.2 [18]. There is no ban on the use of 'brain-dead' donors, but the low level of deceased donor development is primarily due to the cultural characteristics of the local society.

Medical anthropologist Megan Crowley-Matoka offered one of the most interesting explanations of the Mexican phenomenon. In her many years of research, she pushed away from one case that could be considered paradigmatic. The parents of a young man who needed a kidney transplant went to Germany, his mother's homeland. There, both doctors and maternal relatives insisted on a transplant from a deceased donor. However, the family made another decision and returned to Mexico, where the German mother donated her own kidney to her son. This act was not only approved by the Mexican relatives and doctors, but was perceived by all as completely justified and logical: a mother who gave birth to a child should, if necessary, donate part of her organ to the child.

According to the researcher's findings, this logic is closely related to perceptions about sexuality. Since conception is always the result of sexual intercourse and, consequently, the consequence of the mother's moral impurity, childbirth is presented as a moral redemption. The same applies to living donation of a kidney: it is a mother's atonement for carnal sin. Reflecting on this logic, the researcher also turned to the peculiarities of Mexican religiosity, which, in fact, was formed in the 16th century, when ancient Mexico was conquered by Europeans. It was during this period, after the moral humiliation suffered by indigenous Mexicans, that they first adopted the religion of their conquerors, Catholicism. It is noteworthy that the source of the indigenous religious feeling was the image of the Virgin of Guadalupe, which since then has been the most revered Christian symbol in the New World. The image of the Mexican Madonna combined the features of maternal sacrifice with the cultural and religious choices of the Mexicans themselves, who from the mid-16th century became a single nation of indigenous people and their conquerors. In addition, according to Crowley-Matoka, another Mexican woman was deeply rooted in the public mind of the Mexicans, La Malinche, an Indian woman from a noble family, who was given out as a slave to the ruler of the Aztecs, and then presented to them by the leader of the conquistadors Hernan Cortes. By becoming Cortés' concubine, she also became his best spy, helping the Spaniards conquer Mexico. If we bring both of these stories that are important for modern Mexicans to a logical conclusion, the thesis about the naturalness of mother's sacrifice becomes clearer. Every mother in Mexico is first a La Malinche, a traitor to her people, committing a carnal sin with the enemy, and then the Virgin of Guadalupe, mother of the deity, making a redemptive sacrifice [28, 29].

For most Mexicans, the idea of posthumous organ donation is unacceptable. Crowley-Matoka links this to the Mexicans' belief in posthumous resurrection and their belief that the body should remain intact after death, without damage. But along with the arguments relating to the sphere of spiritual culture, according to the researcher, factors of material and cultural order also play an important role. In particular, she noted the poor material infrastructure in Mexican hospitals, the absence of intensive care units in most of them and the apparent lack of ventilators. In addition, the country lacks specialists capable of making reliable diagnosis of brain death.

Crowley-Matoka's work also shows that the naturalness of maternal donation for Mexicans is well aligned with available evidence, although in some cases it may not seem so. In particular, statistics collected by the researcher shows that brothers and sisters are much more likely than mothers to donate to each other. In 12 years of

observation, 168 cases of organ donation by sisters and 160 cases of organ donation by brothers were identified (328 cases in total). In turn, children sacrificed organs for parents 63 times (31 times by daughters and 32 times by sons) while parents donated 76 times for their children (46 times by mothers and 30 times by fathers). Spouses donated organs to each other 42 times – 35 times by wives to husbands and only 7 times by husbands to wives. The same uneven distribution of donor organs between the sexes as between spouses is noted between siblings. Sisters donated 93 times to brothers, while brothers donated half as many, 46 times, to sisters. Siblings were more willing to donate their organs to relatives of the same sex: brothers to brothers 115 times, sisters to sisters 46 times. Despite the fact that all these facts cited by Crowley-Matoka show slightly more sacrifice by Mexican women than by Mexican men [28], we tend to emphasize the main conclusion of all her work: in Mexico, organ donation is a family affair for a variety of reasons. It is because the family is the support for all its members, that is the main consumer of donor organs. Mexicans are not known for extreme individualism. Although some family members willingly donate their organs to others, the point is always to ensure not only the survival of the individual but also the family. All organs are in one way or the other redistributed within the family structure, and there is a moral sacrifice for family survival.

In general, most societies with traditional views, or developing societies, may be considered more willing to accept living organ donation than deceased donation. Unlike Europeans and North Americans who adhere to rationalistic ideas about man, the vast majority of the population in developing countries rejects materialistic views of body and soul. In the West, man is thought of as a sentient being, and the brain is considered the organ of the mind. If the brain dies, then the man dies too. Not the same in traditional societies where brain death is not the death of man at all. In the space of living religious consciousness, death is always something more, a transition to a new state including by the person himself, whose body, even in its postmortem state, is thought to be the sanctuary and property of the Supreme Being.

Turkey

Turkey is a large country where a secular government has been in power for almost a century, and the majority of the population professes Islam, with half in a very moderate form. Its geographical position makes it half a European country, and this remarkably connected with the modernization policy that the authorities have been implementing in various aspects of economic and cultural life. Turkey's experience indicates that the majority of the population is reluctant to allow doctors to use the organs from a deceased donor. Data for 2017 indicate an extremely low level of donor activity for cases of heart, lungs, and pancreas transplantation, i.e. organs that can only be removed from a deceased donor. In contrast, the number of liver transplants from a living donor is quite high – 13.5 persons per million population, and the number of kidney transplants from a living donor is 32.8 persons per million population; this is one of the highest rates in the world (in Europe, only the Netherlands records same figures). Moreover, the level of activity for deceased kidney donation in Turkey is very low – 8.6 (in Europe it is lower only in the Orthodox Greece, Bulgaria, Serbia and Moldova, as well as in Russia) [18].

Special studies show that predominance of traditional views in Turkish society was key to the failures of the national transplant program in the 1990s. Encouraging various forms of modernization, the Turkish government at the same time initiated the creation of a network of private clinics for the middle class and wealthy foreigners, where organ transplants started. This inevitably led to excesses in the practice of acquiring donor organs. As shown in a study by A. Sanal, at the turn of the 1990s and 2000s, clandestine organ trafficking spread in private Turkish clinics, involving some local doctors. Organs were obtained from the bodies of the poor who died in psychiatric hospitals, persons who committed suicide, and victims of major earthquakes. Donor organs were often purchased from the poor, who were specially brought to Turkish clinics from India, Iraq and other places. Assessing the scale of the scandal in Turkish transplant medicine of this time, Sanal calls it a significant scandal. He speaks of these opportunistic Turkish doctors, such as the infamous Dr S (a famous transplant surgeon in the Middle East), sardonically as the "Robin Hood" of Techno-Turkey, acknowledging that they take scarce wealth (organs) from the poor to give to the rich [30, 31]. Turkey, at least until the 2010s, was unable to create a transparent organ donation system, and the population was skeptical of all existing donor practices.

Pakistan

Pakistan is one of the fastest growing countries in Asia. There is also a secular, military-supported government in power and a multimillion-dollar population that professes Islam. Transplantation program, implemented in the country since 1985, is associated exclusively with living organ donation. In the early 2000s, the proportion of patients with severe forms of kidney disease reached 100 people per million population, while about 600–700 operations were performed annually in the country. There is no deceased donation, since transplantation involving the use of brain-dead donors is prohibited by law. At the same time, although Muslim clergy and scholars from Muslim academic centers recognize that deceased organ donation does not contradict Islam, it is rejected and perceived in the mass religious consciousness as an abuse of a dead body. Dominance in the minds of the population

of family-oriented collectivism, which suppresses any autonomy of an individual and his rights does not create a basis for development of posthumous donation. A major transplant center in Pakistan, the Sindh Institute of Urology and Transplantation, has been successfully operating in the country. The organizers were able to adapt the cultural values of Pakistanis to their interests: it carries out kidney transplants only from related donors, and the idea that donation is a moral obligation of every person to a member of his family is actively encouraged. At the same time, major shortage of donor organs is forcing many Pakistanis to look for organs abroad [32–34].

For a quarter of a century now, the medical community, via the WHO and the media, has been persistently talking about global organ shortage, and this shortage does exist where transplant surgeries are performed. Ironically, however, this shortage can come in many different forms. South Asia, primarily India, as well as Sri Lanka and Bangladesh, have long had other kinds of shortages – "shortage is not of donors but of recipients" [35]. A number of recent studies conducted by medical anthropologists indicate that donor practices can be heavily affected not only by the cultural environment, but also by the socio-economic situation.

Bangladesh

Bangladesh is one of the poorest nations in the world. Of its 150 million population, 78% live on less than $2 per day. In the early 2000s, the country took on the sad glory of another "world organ market". Its capital, Dhaka, is a place where the number of people willing to sell their organs (kidney, cornea, part of the liver) is immeasurably greater than the number who are willing to buy them. That is why organ prices tend to decline, while cynical, entrepreneurially-inclined brokers and organ buyers shamelessly deceive the poor who want to sell their organs.

In the mid-2000s, medical anthropologist Monir Moniruzzaman undertook a lengthy study in Dhaka, during which he interviewed 30 local men and 3 women who sold their organs. He also talked with urologists and nephrologists involved in transplants. The information received is nothing short of depressing. Almost all the people who sold their organs were disappointed. In most cases, the buyers did not even pay them the entire promised amount (about US$1,400). After the operation, everyone had a huge scar on their body, which might not have happened if the operation had been done using the laparoscopic method. Almost no one received proper medical care after organ removal. They were forced to return to completely unsanitary conditions after a very quick discharge from the hospital. Most began to have health challenges, as well as major psychological problems. Some soon lost their marriages. None of them could use the money to at least somehow improve their lives, and many of them did not even have enough to cover all debts. Donors who sold their kidneys invariably remembered the day of their operation as the darkest day of their life. One of those interviewed by Moniruzzaman said that he feels "only half human" after the surgery [36, 37].

The national law on organ transplantation in Bangladesh was adopted in 1999, and according to this law, organ trafficking is officially prohibited in the country. Criminals face a hefty fine and a jail term for any violation. In reality, however, no one is punished for this. Besides, the country's five largest newspapers regularly feature adverts by buyers ready to buy an organ. In Monizzaman's view, even the term "donation" itself seems inappropriate for Bangladesh. People don't give organs here; they sell them out in the open. All this is not only a consequence of the poverty of the vast masses of the population, but also of what the anthropologist calls "bioviolence." The richest organ buyers prefer to take their "donors" abroad, usually to India or Singapore, and already have an operation there. Those who are not so wealthy use the services of local hospitals, such as the Sheikh Mujib Medical University Hospital in Dhaka. The wealthiest fly to the US, bringing with them the purchased organ. The altruistic philosophy, warmly endorsed by the world medical community, according to which the life of some people can be saved by the lives of others, looks completely different here: the lives of the rich are extended at the expense of the lives of the poor. Scheper-Hughes' work shows how the global flow of living donor organs follows the modern route of capital: from poor countries to rich countries, from South to North, from Third to First World; there is a kind of "medical apartheid" [38, 39].

CONCLUSION

The concept of "organ shortage" has firmly entered the professional discourse of transplantation medicine and became a peculiar reflection of the current crisis of the philosophy that underlies the modern transplantation practice. This is the philosophy of altruism, which was first developed back in the 19th century by French philosopher Auguste Comte. Guided by the philosophy of altruistic donation, transplantation medicine representatives call on other members of society to share the ideals of this philosophy – volunteerism, gratuity, solidarity, etc. However, the situation with the global organ shortage clearly indicates that consensus is not always reached between professionals and society on this issue. Society does not necessarily accept the philosophy (ethics) of altruistic donation, at least not in its entirety. This fact necessitates a more thorough study of the society's attitude towards organ donation. In this regard, the global problem of organ shortage cannot be seen only as a problem for the professional medical community. It requires

an interdisciplinary research, joint work among doctors and humanities scientists.

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TRANSPLANTATION TECHNOLOGIES FOR TREATMENT OF CARBOHYDRATE METABOLISM DISORDERS

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The review includes results of retrospective and prospective clinical studies (foreign and national) and guidelines on the use of transplantation technologies for treatment of type 1 diabetes and pancreatogenic diabetes in chronic pancreatitis and pancreatic conditions. Modern data on prevalence of diabetes and modern insulin delivery methods are presented. Results of transplantation of pancreas and islets of Langerhans in primary insulin-dependent conditions are considered. Analysis of the technology for isolation and autotransplantation of islets after pancreatectomy in chronic pancreatitis and benign tumor diseases are given.

Keywords: pancreas, type 1 diabetes, pancreatogenic diabetes, pancreas transplantation, islets of Langerhans.

INTRODUCTION

Pancreatic conditions are associated with type 1 diabetes (T1D) and pancreatogenic diabetes mellitus (PD). T1D is characterized by autoimmune destruction of insulin-secreting cells resulting in absolute insulin deficiency. This disease is a significant medical and social problem for several reasons. Cases of T1D are rising every year. In Russia, 3.12% of the population (4,584,575 million people) were diabetic as of January 1, 2019. About 256,200 of these had T1DM. Currently, the average prevalence of T1D in Russia is 174.4 per 100,000 population. In 2018, a total of 10,805 new T1D cases were detected [1]. The vast majority of patients are children and young people under the age of 30. Carbohydrate metabolism disorders over time lead to acute fatal or chronic disabling diabetes complications [2]. Estimates from the Federal Diabetes Registry show that in T1D, 33.6% of patients developed diabetic polyneuropathy, 27.2% – diabetic retinopathy, 20.1% – nephropathy, 12.1% – diabetic macroangiopathy, 4.3% – diabetic foot syndrome, 3.5% – coronary heart disease, 1.5% – cerebrovascular disease, and 1.1% had myocardial infarction. Indicators presented are determined by the data on number of patients that visited the hospital. With active screening, incidence of such complications will certainly increase [3]. T1D patients have limited adaptability and self-actualization. Treatment requires huge expenses on expensive drugs and self-monitoring devices. Optimistic analysis based on the Russian sample showed that the average annual cost per patient with T1D was 81,100 roubles. The cost of treating patients with existing chronic complications and not reaching the target levels of glycated hemoglobin is much higher than in patients without complications with compensated diabetes [4]. There is no doubt that preventing or slowing down the progression of diabetes complications can be achieved through adequate long-term control of glycemia levels. Lifelong insulin therapy remains the only treatment available. Despite the emergence of pharmacokinetically more adapted insulin drugs, individual self-monitoring blood glucose devices (glucometer and continuous real-time monitoring systems) and insulin injection devices (insulin syringes and insulin pumps), stable glycemic parameters are not always achieved. Fluctuations in blood glucose levels, episodes of hyperglycemia and hypoglycemia are observed in almost all patients. This is due to differences in insulin requirements depending on diet, physical activity and many other factors that are difficult to foresee or control. Creation of a closed loop "artificial pancreas" based on inverse correlation between current blood glucose levels and the insulin dose administered is only in the clinical trials phase.

Pancreatogenic diabetes (PD) is a consequence of the loss of pancreatic parenchyma resulting from chronic relapsing pancreatitis, pancreatic necrosis, and partial or total pancreatectomy. This review focuses on patients who have undergone pancreatectomy for painful chronic relapsing pancreatitis or benign pancreatic tumors. Unlike T1D, which selectively destroys beta cells, PD is characterized by a lack of not only insulin, but also other islet hormones regulating glucose metabolism rates. Despite rare development of ketoacidosis and moderate hyperglycemia, these patients are prone to brittle diabetes with high variability of glycemia and repeated

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severe hypoglycemia, which reduces their recognition. Incidence of chronic micro- and macrovascular complications in T1D and PD is the same.

The use of transplantation technologies may be a promising option for replacing lost insulin-producing function. Achieving a euglycemic state will allow patients with pancreatic conditions to avoid the negative impact of hyperglycemia – the trigger mechanism for complications – and, most importantly, reduce the likelihood of developing severe, sometimes fatal, hypoglycemic conditions [5].

ANATOMICAL AND PHYSIOLOGICAL RATIONALE FOR THE USE OF TRANSPLANT TECHNOLOGIES

The human pancreas is a glandular organ that includes the exocrine and endocrine parts.

The exocrine part of the pancreas is represented by pancreatic acini and the excretory duct system.

The endocrine part of the pancreas is formed by pancreatic islets lying between the acini (islets of Langerhans). Islets of Langerhans contain 20–25% glucagon-producing alpha cells and 75–80% insulin-producing beta cells, somatostatin-producing D cells, VIP (vasoactive intestinal polypeptide) cells and PP (pancreatic polypeptide) cells. With age, there is a change in the pancreas between its exocrine and endocrine parts – the endocrine component (number of islets) reduces.

The pancreas has about 10,000,000 islets, which are compact clusters of secretory cells arranged in bunches or cords. Cells surround the capillaries of the islets in layers, being in close contact with the vessels.

TRANSPLANTATION TECHNOLOGIES

1. Pancreas transplantation

Transplantation of beta cells as part of an organ or isolated as a cell suspension is a pathogenetically justified method of T1D treatment. Despite many animal experiments, the first successful pancreas transplant was performed simultaneously with a renal graft to treat a T1D patient in 1966, at the University of Minnesota, and was conducted by William Kelly and Richard Lillehei. Until 1980, the operation was considered experimental. Active interest in pancreas transplantation returned in the late 1970s amid improved immunosuppressive therapy and surgical techniques [6].

In Russia, simultaneous kidney-pancreas transplantation with grafts obtained from a deceased donor was first performed by Valery Shumakov in 1987 [7]. Successful transplantation of a gland fragment from a living related donor was performed by Sergey Gauthier [8].

There are 44 transplant centers in Russia, but only four of them perform pancreas transplantation. In 2018, 17 pancreas transplants were performed [9].

Important factors for successful functioning of a pancreatic graft include recipient selection, assessment of the donor and the donated organ. Primary selection is based on blood group compatibility and a negative cross-match response. The number of HLA matches is important for long-term graft functioning [10].

Based on the outcome of 445 transplant surgeries, A. Gruessner et al. showed that donors older than 45 years and obese donors are a significant factor for such complications as major vessel thrombosis, intra-abdominal infection, and failed pancreatodigestive anastomotic failure [11]. Similar data are demonstrated by domestic authors [12].

Absolute contraindications for pancreas transplantation include functional disorders of the cardiovascular system, such as low cardiac output fraction, unstable coronary heart disease, mental diseases, noncompliance, active infection, and malignant tumor [13].

Diabetic nephropathy is the main criterion determining the transplantation option to choose (isolated or simultaneous). Less than 40 mL/min reduction in creatinine clearance, or dysfunction in the previous renal graft is an indication for simultaneous kidney-pancreas transplantation. Isolated pancreas transplantation is indicated for T1D patients without severe nephropathy. Prevalence of threat to life with complicated diabetes (severe hypoglycemia) over the possible consequences of prolonged immunosuppression is an obligatory criterion for selection.

For T1D patients with a previously transplanted kidney, subsequent pancreas transplantation is justified in terms of preventing transplant nephropathy and improving the quality of life. Also, an argument in favor of pancreas transplantation after kidney transplantation is the immunosuppression protocol formed and established by this time, which promotes optimum physiological and psychological adaptation of the patient to the upcoming surgery. The necessary condition for pancreas transplantation is stable function of the previously transplanted kidney (creatinine clearance >50 mL/min).

The technical aspects of performing pancreas transplantation at various transplantation centers follow the same principles: ensuring adequate arterial blood supply to the pancreas and duodenum segment, free venous outflow from the transplant and ensuring pancreatic exocrine secretion [14].

Thanks to advances in transplantation technologies and immunosuppression regimens, graft and recipient survival rates have significantly increased. Simultaneous liver-kidney transplant in diabetic patients significantly increases kidney graft and recipient survival in comparison with isolated kidney transplantation [15].

Recipient survival at one year post-transplantation is above 95% for pancreas transplants alone, and 90% at 3 years post-transplantation. One-year graft survival is

85% in combined pancreas/kidney transplant compared to 79% in solitary pancreas transplants [16] and 78–83% in pancreas transplant after kidney transplant [17].

Thus, simultaneous pancreas/kidney transplant is an effective method of treating patients with T1D complicated by end-stage renal disease.

2. Pancreatic islet transplantation

A significant limitation to higher numbers of pancreas transplantation is the unsatisfactory state of pancreas from deceased donors. Moreover, there are no objective criteria for assessing organ complex quality. According to reports from the German registry, three quarters of the reasons for graft rejection are subjective [18].

In case of rejection of whole-organ transplant, technologies have been developed for isolating islet cells for subsequent transplantation to patients with insulin-deficient carbohydrate metabolism disorders.

In 1977, the first successful islet cell autotransplantation (ICT) was performed in Minnesota.

In a number of countries (Canada, Australia, Great Britain, Switzerland, Norway, etc.), islet cell transplantation is a medical care standard "that does not require further scientific justification" [19].

Until 2000, according to the world registry, only 12.5% of patients had euglycemia after pancreas transplantation for more than 1 week, and only 8.5% of patients retained graft function after a year.

In 2000, a paper by Professor Shapiro with co-authors from Edmonton reported that all 7 patients, who underwent ICT according to the implemented protocol, attained sustained insulin independence from 6 to 12 months [20]. The main points of the "Edmonton Protocol" remain generally accepted nowadays:

1. Thorough selection of recipients. The main group of patients who are shown to have the so-called brittle diabetes, characterized by hard-to-control glucose levels, while constant fluctuations from severe hyperglycemia to critical hypoglycemia significantly accelerate patient disability and reduce their lives considerably.
2. Very high dose of islet cell suspension. The standard rule before the Edmonton Protocol was introduced was: 1 donor – 1 recipient. The dose recommended by the protocol is at least 10,000 IEQ/kg with additional administrations in the case of reduced function, which requires the use of 2 to 3 grafts per recipient.
3. Modern immunosuppression regimens. Nonsteroidal therapy, as well as induction with IL-1 and IL-2 inhibitors, showed excellent results in ICT.

Currently, the clinic at the University of Alberta in the Canadian city of Edmonton continues to be the leading research institution in the field of ICT for T1D. The best ICT outcomes are achieved by centers adhering as closely as possible to the standards established in Edmonton [21].

According to the above study, among the 48 study subjects, the primary end point was successfully met by 87.5% at 1 year and by 71% at 2 years. Two years after ICT, the median HbA_{1c} level was 5.6%. No ICT-caused death has been reported. Data were obtained on 50% of the 5-year ICT efficiency in a number of centers, which brings the results closer to whole-pancreas transplantation. Of the current advances that have significantly improved ICT outcomes, the use of alemtuzumab (anti-CD52 antibody) induction and etanercept (TNF inhibitor) to suppress the inflammatory response after ICT should be noted.

Biologically active islet encapsulation in a porous peptide-fragmented alginate structure is a promising technology. Pores allow cells to receive oxygen and nutrients and to freely secrete hormones into the environment. The capsule protects the islets from exposure to immunocompetent cells, which eliminates the need for immunosuppressive therapy and prevents surrounding fibrosis. There is an active search for ways to supplement the extracellular matrix of the pancreas, stem cells, oxygen nanotransporters inside the capsule to increase the efficiency and duration of its functioning.

Donor phase (ICT)

The number of islets obtained, and their quality, largely depend on age, BMI (body mass index), donor, and cold ischemic time.

Most centers routinely examine donors with increased BMI for impaired glucose tolerance, since it is known that obesity is often associated with type 2 diabetes.

Prolonged cold ischemic time lasting for more than 6–8 hours has a negative effect on the dose and quality of the obtained islet cell isolate. It is preferable to use a UW solution for preservation, which does not exclude the use of an HTK solution.

According to some research, it is easier to get adequate dose of cellular isolate in age-related donors (51–56 years old) (83% versus 37% in donors aged 19–28 years), but the secretory capacity of these islets is much lower. Donors in the young age group are considered to be "ideal", but the technically more complex isolate preparation procedure due to the severity of the glandular fibrous structures should be taken into account.

Immediately after introduction of islet cells, 70–80% of their mass turns out to be non-viable even if strict donor selection criteria similar to those for a whole-organ transplant are met [22].

The latest technological advances of the time were used in isolating islet cells at Edmonton:

– splitting of the pancreas using the latest generation of collagenase enzymes (liberase)

– cellular isolation by the automatic method proposed in 1988 by Professor Ricordi. This method allows to minimize cell injury and significantly increase their concentration at the output and the degree of purity of the isolate.
– use of a computerized cell separator, which allows to get rid of fragments of the stroma, exocrine cells, etc from the isolate.

Today, stringent requirements for preparation of ICT materials in a number of countries have led to the creation of single laboratories serving multiple centers to maintain high GMP standards and save resources.

Islet isolation technique

Islet isolation and preservation method begins already during explantation (minimal injury, rapid systemic and local cooling of the pancreas).

The purpose of the pancreatic processing stage is to free the islets from the surrounding extracellular matrix. This is achieved by a combination of mechanical and enzymatic "digestion" of the gland. First, external fat is removed, while special attention is paid to preserving the integrity of the organ capsule. Next, the pancreatic duct is cannulated and a collagenase solution is injected, which allows for 10 minutes to cause swelling of the gland and to separate the islets from the surrounding exocrine tissue. Areas of pancreas that have not been exposed to overstretching are not suitable for further processing. Then the pancreas is cut into several parts and placed in the Ricordi chamber. This closed system maintains constant recirculation of the warm solution containing collagenase, and with the help of hollow metal balls, the gland tissue is mechanically fragmented and filtered through a screen (with 500 μm pores). If the digestion process does not stop after most of the islets have been released, they are rapidly damaged by collagenase. Currently, the most commonly used mixture is Roche's Liberase HI. A disadvantage of this mixture is that it uses clostridial collagenases, and although pathogen transmission risks are negligible, such concerns exist. Alternative mixtures, including those with the ability to regulate collagenase activity, are being tested.

After dissociation of the islets, they must be purified. It is known that introduction of large amount of isolate into the portal vein leads to serious complications up to lethal (thrombosis, embolism). Islet purification is based on the difference in the density of islet and exocrine cells. When placed in a medium with a known density and centrifugation, islet cells, as less dense, occupy the upper layer of the medium. Only a fully automated centrifuge-type separation system can obtain a fraction of islet complexes with high degree of purification (≥70%) [23].

Purified islets are counted in islet equivalents using automatic counters, where 1 equivalent is equal to an islet with 150 μm diameter. Microscopy is performed to assess islet viability. Functionality is assessed using insulin tests, as well as by injecting diabetes into mice. Assessment in the mouse model has the highest correlation with the clinical effect of ICT, but it takes a lot of time and is almost never used at present. Sterility is established by testing for aerobic and anaerobic bacterial cultures, and for mycoplasma and endotoxin [24]. According to the classic Edmonton protocol, the isolate was injected immediately after preparation. However, storing the cell culture for a certain time allows to optimize the logistics (recipient preparation, immunosuppression induction, isolate transportation) and reduce immunogenicity in the medium.

In case of incomplete response, additional infusions are carried out, which requires additional donors for each recipient [25].

Administration technique

Some islet injection locations were studied: under the kidney capsule, in the greater omentum, the anterior chamber of the eye. However, intraportal administration of islet cells is today the standard method in clinical practice. It is minimally invasive and safe. Bleeding and portal thrombosis – the most formidable complications of this operation – occur with a less than 10% frequency and are very rarely fatal.

Islet response to implantation

Positron emission tomography was used to established that immediately after intraportal administration, 50–70% of the islets lose their viability. Therefore, the use of suspension from 2 to 3 donors is necessary [26, 27].

The main damage to islets after administration occurs due to pathological processes developing in the recipient's body. The most studied consequence of intraportal administration of islets is instant blood-mediated inflammatory reaction (IBMIR), which is an immune response developing immediately after transplantation from blood clot formation, and infiltration by mast cells and macrophages [28, 29]. Microthrombi consisting of platelets, neutrophils, and monocytes appear 5 minutes after islet infusion [30, 31]. The response is initiated by a coagulation cascade, which peaks at 6–12 hours after islet infusion [32].

Complementary activation also occurs. Inside and on the surface of islets, C1q, C4, C3, nad C9, IgG, and IgM are determined, which leads to formation of anaphylotoxins C3a and C5a. A set of cytokines stimulates migration and activation of inflammatory cells. Activated thrombin causes endothelial cells to secrete adhesion factors, such as P selectin, resulting in platelet aggregation. Endothelial cells secrete pro-inflammatory interleukins IL-6 and IL-8, which help migrate neutrophils and ma-

crophages into the focus. Monocytes and macrophages help maintain an inflammatory response.

Islet cells undergoing stress caused by hypoxia and injury during isolation provoke inflammation by TF secretion and expression of proinflammatory factors: HMGB, IFNc, IL-6, IL-8, IL-1b, IFNc-induced protein, MCP, tumor necrosis factor (TNF), nuclear factor kappa B (NF-κB), nitric oxide, and others [33, 34].

The search for ways to reduce inflammatory response showed that heparin and low molecular weight dextran sulfate have positive effect. The use of other drugs – nicotinamide, thrombin inhibitors, sCR1 complement inhibitors, C5a inhibitors – is being investigated. Alternative ways of protecting islets by PEGylation and mast cell coating are being studied. In clinical practice now, only heparin is being used routinely and widely [35, 36, 37].

Ischemia-reperfusion injury (IRI) of the islets is difficult to characterize due to lack of possibility of biopsy. Its degree can be indirectly detected and evaluated by transient increase in AST and ALT levels, which is observed in half of the recipients and peaks by the end of the first week after ICT. The systemic effect of IRI after ICT is weakly expressed, but locally it significantly promotes early loss of islet viability [38, 39]. Native islet cells oxygenate very well, consuming 5–15% of oxygen flowing through the pancreas, with about 40 mmHg oxygen tension. Under culture, large islet complexes suffer from hypoxia, which causes central necrosis and apoptosis. During the first days after intraportal infusion, islets are oxygenated only by diffusion in the low oxygen tension portal system, which is exacerbated by coagulation cascade in IBMIR. It takes 7 to 14 days for an autonomous functional blood supply system to be developed using newly formed capillaries. Even after 3 months, oxygen tension does not exceed 5 mmHg. Moreover, hypoxia does not depend only on intraportal location of the islets; studies on introduction of islet cells into more blood-supply areas showed similar outcomes [40, 41].

There is evidence of the positive effect of cycles of blocking and restoring portal blood flow (ischemic preconditioning), which has a protective effect on both the liver and islets.

Immunosuppression in islet cell autotransplantation

Calcineurin inhibitors (cyclosporin and tacrolimus) and steroids (prednisone) were some of the standard immunosuppression regimens used in the late 90s and early 2000s, in fairly large dosages. These drugs provide effective prevention of graft rejection, but have a number of side effects, including toxicity to islet cells. The Shapiro team successfully applied a steroid-free immunosuppression regimen with reduced tacrolimus dose through daclizumab (antibodies to interleukin receptors) induction and addition of sirolimus (proliferation inhibitor) to the treatment protocol.

ICT performance assessment

There is now a paradigm shift in measuring ICT effectiveness as experience is gained. Previously, ICT goal was to achieve and maximize the duration of insulin independence. Currently, ICT is considered a treatment for insufficient beta cell function, regardless of the cause, if the patient has brittle diabetes with problematic hypoglycemia or hyperglycemia, despite optimized medical care. The use of insulin after pancreas or islet transplantation does not indicate a loss of graft function. To maintain glycemic levels, patients may need low doses of exogenous insulin, normalizing blood glucose levels that can be achieved when part of the insulin requirement is supplied endogenously from a functioning graft.

The optimal function of the beta-cell graft is determined by the presence of an almost normal glycemic profile, estimated by the level of glycated hemoglobin (HbA1c) 6.5% or less, absence of severe hypoglycemia, lack of need for exogenous insulin and increased C-peptide level compared with the pre-transplantation level.

A good beta-cell graft function reduces daily insulin demand by 50% (should be <0.5 IU per kg of body weight per day) provided that blood sugar level is adequately controlled (HbA1c <7%) and increase in C-peptide (should be at least 0.5 ng/mL) compared with pre-transplant levels.

The borderline function of a beta-cell graft is determined by inability to reach the target HbA1 level of less than 7.0%, by occurrence of any severe hypoglycemia, or by a less than 50% decrease in insulin demand, despite increased C-peptide level compared to the pre-transplantation level.

If reduced hypoglycemia awareness, frequent severe hypoglycemia or severe glycemic lability, which improved after transplantation, has been documented prior to transplantation, then it may be appropriate to consider the beta-cell graft as having a clinical effect. Clinically, the benefits of maintaining and controlling beta-cell graft function may outweigh immunosuppression risks.

In the absence of evidence of clinical improvement, even with increased quantitative level of C-peptide after surgery, borderline and insufficient beta-cell graft are considered clinically unsuccessful.

Currently, over 60,000 pancreas transplantation and 4,000 ICTs have been performed worldwide. Comparative characteristics of the procedures are presented in the table below.

Thus, ICT technology, with careful adherence to protocol, is good for correcting insulin-dependent carbohydrate metabolism disorders, preventing severe hypoglycemia. In terms of efficiency, it is practically not inferior to

Table

Comparative characteristics of pancreas and islet of Langerhans transplantation

Generalized experience	Pancreas transplantation	Islet cell transplantation
	Over 60,000	Over 4,000
Insulin independence 1 year 5 years	 90% 70%	 60–80% 25–50%
Function (C-peptide 5 years)	70%	70%
Best combination options	SPK > PAK > PTA	SIK, IAK, ITA equivalent
Intervention	Extensive laparotomy	Interventional radiology
Complications	Severe	Rare
Mortality	4–6%	none

whole-organ transplantation. The ICT technology gives higher safety and accessibility.

Pancreatogenic diabetes mellitus

After critical reduction in the mass of pancreatic parenchyma, pancreatogenic diabetes mellitus (PD) develops, which differs from T1D. Total pancreatectomy, pancreatic necrosis, chronic fibrosing diseases, gland atrophy due to chronic inflammation, and tumors may be the reasons for the loss of critical islet mass.

The peculiarities of PD are determined by the lack of function not only of beta-cells, but also of the rest of the endocrine cells of the pancreas. Pancreatic polypeptide deficiency leads to hepatic insulin resistance and increased liver glucose production. However, under endogenous hyperinsulinemia, sensitivity of peripheral tissues to insulin increases, which helps to reduce blood glucose. Lack of glucagon secretion and impaired secretion of intestinal incretins also reduce its level, increasing the risk of hypoglycemic conditions [42]. Episodic hypoglycaemia was experienced by 79% of patients, while 41% experienced severe hypoglycaemia with loss of consciousness [43].

Despite rare development of ketoacidosis and moderate hyperglycemia, PD patients are prone to a labile course with high glycemic variability and repeated severe hypoglycemia. Incidence of chronic micro- and macrovascular complications is the same for T1D and PD.

One of the limiting factors in planning a pancreatectomy is doubts about patient compliance and commitment to subsequent lifelong PD treatment.

Islets autotransplantation after total pancreatectomy

Currently, the ICT procedure, due to its safety, can be considered as a tool for correcting carbohydrate metabolism disorders in planning operations related to total removal of the pancreas (pancreatectomy).

Pancreatectomy is indicated for patients with irreversible common pancreatic diseases. There have been published works on the use of auto-ICT after removal of the pancreas for benign tumors, injury and arteriovenous malformations. The possibility of auto-ICT in ductal adenocarcinoma and intraductal papillary mucinous neoplasm is currently controversial and requires further study [44].

Islet cell autotransplantation in painful chronic pancreatitis

The technology for treatment of chronic pancreatitis pain – total pancreatectomy followed by auto-ICT – is actively developing. With autotransplantation, there is no need for an immunosuppression protocol, thus excluding the negative effects of immunosuppressive therapy after ICT.

Performed for the first time in 1977 by Sutherland et al (University of Minnesota, USA), pancreatectomy with islet cell autotransplantation allowed the patient to get rid of pain and stay in a state of euglycemia for 6 years, after which he died from causes not related to the underlying disease. Such a result aroused great interest in the world and to date, there have been more than a thousand operations [45]. The main patient population for the TPIAT procedure includes people with painful chronic pancreatitis who need constant pain relief.

Prevalence of chronic pancreatitis (CP) is quite high. Annually in the USA, depending on the region, 4–12 new cases are detected per 100,000 population. In Europe, prevalence of chronic pancreatitis ranges from 4 to 40 cases per 100,000 population [46, 47, 48]. In Russia, it is up to 30 new cases per 100,000 population [49].

Idiopathic pancreatitis is the second most common and it is mainly caused by genetic conditions – associated with mutations in the PRSS1, CFTR, SPINK1, and CTRC genes [50, 51].

The most pronounced clinical manifestation of CP is constant or intermittent pain in the upper abdomen, which is observed in 85–90% of patients, It leads to significant deterioration in quality of life, up to constant prescription of narcotic drugs [44, 52, 53].

In conservative management of CP patients, the drugs of choice are analgesics, predominantly opioid-based. They suppress pain well, but cause dependence, and, with prolonged use, lead to many serious side effects. In USA, where opioid therapy is most common, 26,000 deaths from the effects of opioid prescribed by doctors are recorded annually [54].

Total pancreatectomy with autotransplantation of pancreatic islets is most effective in patients with unexpanded pancreatic duct and in patients with hereditary pancreatitis [55].

Most researchers agree that, if there are indications, the operation should be performed as soon as possible. Previous pancreatic drainage surgeries and a long course of pancreatitis significantly affect the received dose and quality of islet cells [56].

This treatment method is based on total removal of the pancreas as a source of persistent pain with subsequent islet autotransplantation, most often into the portal vein [57]. Such surgical interventions have been steadily increasing in number recently [58, 59, 60]. The vast majority of centers show zero mortality after surgery; it does not exceed 1% in the general analysis [61, 62]. The outcomes of such interventions are evaluated on the basis of changes in the quality of life: disappearance of pain and reduced need for opioids, prevention of hypoglycemia. Achieving insulin independence is not an end in itself [63].

The number of patients who got rid of drug dependence after pancreatectomy with islet autotransplantation varies from 35 to 100% (on average above 60%) during a 12–24-month follow-up. The remaining patients noted significant reduction in opioid dosage and transition from daily to episodic pain medication. Analysis of the pain scale showed a change from 60–100 (out of 100) points to 8–20 (100) within 1 year. This effect may persist for a long time. Approximately 73% of patients remain independent of analgesics for more than 5 years [61].

Despite the fact that insulin independence is not long-term stable in 15–41% of patients, euglycemia continues for 6–12 months.

Currently, it is believed that surgery is indicated for patients who fall under the following five criteria [62, 63]:

1. Chronic pancreatitis with pain lasting for more than 6 months amid one of the following symptoms:
   - Presence of pancreatic calcifications in CT.
   - At least two of the following symptoms: 4 or more criteria out of 9 according to endoscopic ultrasonography; changes in the pancreas duct and pancreatic parenchyma in magnetic resonance cholangiopancreatography; changes in the endoscopic pancreatic function test (peak value of Hco2 ≤80 mM).
   - Diagnosis of chronic pancreatitis confirmed by histopathological examination.
   - Appropriate history and documented hereditary pancreatitis (PRSS1 gene mutation).

   Or
   - Past history of recurrent acute pancreatitis (more than one episode of an attack of characteristic pain in combination with changes in instrumental studies and/or a three-fold or more increase in serum amylase or lipase).
2. One of the following symptoms:
   - Daily need for narcotic analgesics.
   - Decreased quality of life associated with pain (inability to attend school, repeated hospitalizations, inability to perform activities appropriate to age).
3. Currently confirmed or untreated pancreatitis without obvious cause.
4. No effect from drug therapy and endoscopic treatment methods.
5. Adequate islet functioning (no diabetes or positive C-peptide).

Patients with diabetes on the background of negative C-peptide, who meet criteria 1 to 4 are shown to perform total pancreatectomy without autotransplantation.

The following are considered as relative contraindications [58]:

1. Existing T1D or PD.
2. Steatohepatitis.
3. Portal vein thrombosis.
4. Portal hypertension.
5. A past history of longitudinal pancreatico-jejunostomy.
6. Visceral hyperalgesia.
7. Psychological disadaptation.

When evaluating a candidate for pancreatectomy with islet autotransplantation, it is necessary to take into account that age-related changes, alcohol, smoking, diabetes and obesity can cause fatty degeneration and pancreatic atrophy in combination with pain under the mask of chronic pancreatitis [64]. At the same time, prolonged use of narcotic analgesics can lead to functional changes in the intestines and central nervous system, which are difficult to diagnose and treat, but can affect surgical outcomes [65, 66]. It has been proven that long-term outcomes of surgical treatment of patients with hereditary chronic pancreatitis are significantly better than in patients who abuse alcohol [75, 76].

An important factor in preoperative examination is the evaluation of the endocrine function of the pancreas even in the absence of confirmed diabetes. Glucose tolerance test is easily reproducible, but its results do not correlate with the volume of the unaffected islet apparatus [69]. More effective for indirect estimation of the volume of functioning islet apparatus is a method for assessing the secretion of insulin and C-peptide induced by arginine [70].

Treating a patient with chronic pancreatitis is very expensive [71]. Moreover, studies conducted in the UK showed the cost-effectiveness of total pancreatectomy with islet transplantation in comparison with traditional methods of treating chronic pancreatitis [72].

A currently adopted multicenter clinical protocol for islet transplantation named “07”, includes the following

components necessary in the postoperative period after autotransplantation [73]:
- Timoglobulin.
- TNF-alpha inhibitor (etanercept).
- Heparinization.
- Insulin therapy for 8 weeks of the perioperative period.
- Tacrolimus and sirolimus as in the Edmonton Protocol [74, 75, 76].

Pancreatectomy with islet autotransplantation improves quality of life in patients. Most patients get rid of severe pain. Various authors have reported that up to 79% of patients do not need narcotic analgesics after surgery [63, 77]. In addition, patients do not require insulin therapy in a significant number of cases in a long-term postoperative period [78, 79].

CONCLUSION

Type I diabetes and pancreatogenic diabetes mellitus are a huge social problem around the world. The only available massive way to control blood sugar levels is by administering exogenous insulin. Improving insulin therapy, creating new convenient and genetically "close" insulins, and pump therapy remain only a symptomatic treatment that has certain shortcomings, such as disabling complications and fatal hypoglycemia.

Transplantation technologies for the treatment of severe insulin-dependent carbohydrate metabolism disorders are promising due to their high efficiency and safety. The advantage of using transplant technology is down to the delicate biological inverse relationship between serum glucose levels and insulin production by beta cells.

General shortage of donor organs and insufficient quality of received pancreas for transplantation necessitate development of ICT technology. Analyses of whole-organ transplantation and islet suspensions show similar efficacy with greater safety of the ICT procedure. The first achievements in the field of bioactive islet encapsulation give the procedure significant advantages – no immunosuppressive therapy. Encapsulation also allows for long-term functional activity of the islets.

In the Russian Federation, actions are required to legally legitimize the ICT procedure (introduction of islets of Langerhans in the list of organs and tissues for transplantation).

The technologies of allo- and autotransplantation of cell cultures of pancreatic islets are similar and are derivatives of the same protocol. It is advisable to create specialized and certified laboratories for isolation and storage of islets. The technical features of performing pancreatectomy do not present difficulties for doctors at a specialized pancreatological center. Implementation of a pancreatectomy protocol with islet autotransplantation will improve treatment outcomes for a large group of patients with chronic pancreatitis.

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FIBRIN – A PROMISING MATERIAL FOR VASCULAR TISSUE ENGINEERING

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This review looks at the use of fibrin in vascular tissue engineering (VTE). Autologous fibrin is one of the most affordable biopolymers because it can be obtained from peripheral blood by simple techniques. A description and comparative analysis of the methods and approaches for producing fibrin gel is provided. The ability of fibrin to promote cell attachment and migration, survival and angiogenesis, to accumulate growth factors and release them in a controlled manner, are unique and extremely useful in VTE. Fibrin gels can serve as a three-dimensional matrix molded in different sizes and shapes to be applied in a variety of ways, including as a scaffold, coating, or impregnation material. Fibrin's high porosity and biodegradability allows controllable release of growth factors, yet fibrinolysis must be tightly regulated to avoid side effects. We discuss the main methods of regulating the rate of fibrinolysis, as well as possible side effects of such exposure. Low mechanical strength is the main limitation in using fibrin as a scaffold for vascular tissue engineering. Possible options for increasing the strength properties of fibrin matrix and evaluating their effectiveness are presented. We propose that unique biocompatibility and ideal biodegradation profile of fibrin justify its use as a scaffold material for developing an ideal fully autologous small-diameter tissue-engineered vascular graft.

Keywords: vascular tissue engineering, fibrin, cell carrier, biopolymer, autologous tissue-engineered vascular graft, fibrinolysis, mechanical strength, implantation.

BACKGROUND

Active search for an ideal material to be used in vascular tissue engineering (VTE) is continually going on. Developed synthetic materials, such as dacron or polytetrafluorethylene (PTFE), polyethylene terephthalate (PET) are successfully used in large diameter vessel reconstruction, however their applicability for creating low diameter vascular prostheses (less than 6 mm) is low because of intima hyperhrophy and thrombosis due to incomplete endothelization, low blood flow velocity and mismatch in compliance (vessel diameter and its adaptive reactions to arterial blood pressure changes) [1]. Lack of effective low diameter vascular grafts facilitates the search for innovative strategies, new materials and their modifications.

Understanding the significance of normal physiological reactions of the vascular wall in the prevention and control of thromboses, hyperplasia and inflammation created a whole new area in VTE devoted to imitating the structure and function of the native arterial wall while developing new generation vascular grafts. Among the most potentially advantageous approaches in VTE related to maximal imitation of the extracellular matrix (ECM) in combination with biological functionality of the product, biogel cell colonization and the technology of biodegradable frames should be noted. In these areas fibrin presents a special interest for researchers, as it possesses a set of unique characteristics which make it a practically ideal natural biological material for VTE [2].

Fibrin is formed at the final stage of the coagulation cascade and presents a natural biodegradable matrix. Fibrin-based biomaterials are ideally biocompatible, possess high affinity to various biological surfaces, controlled biodegradation by means of fibrinolysis, the biodegradation products of which are non-toxic [3].

Currently fibrin gel is actively used at the clinic for hemostatic purposes, closure of the wound surface and as a sealant. Search is going on in tissue engineering for fibrin matrixes in ophthalmology (sclera [4] and lens [5] reconstruction), in neurology (plexus and peripheral nerve reconstruction [6]), in traumatology and orthopedics (cartilage reconstruction [7]), during artificial skin development [8], etc. Being a natural physiological frame, it supports angiogenesis and tissue reparation [9]. Fibrin fibers contain cell adhesion sites which create a platform for cell adhesion, migration and proliferation, as well as conditions for adequate tissue formation [10]. The 3D porous structure of fibrin supports cell migration into the frame and their vital activity due to nutrient and oxygen diffusion into the fibrin frame and waste removal [11]. Fibrin is the most available of all polymers. Fibrin gels can be easily and promptly obtained from the patients' own blood. Autologous scaffolds formed on its basis do not have the risk of transferring various pathogen transfer or launching the immune reactions of the organism to

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a foreign body. The properties and special features of fibrin which have crucial significance for VTE will be described further in more detail.

MECHANISMS OF FIBRIN GEL FORMATION

Fibrin is a product of fibrinogen polymerization as the final stage of the coagulation cascade. Fibrinogen is a dimer of glycoprotein which consists of 3 pairs of identical chains (α, β and γ). At the first stage active single molecules of fibrin monomer are formed, facilitated by thrombin, which are capable of polymerization and forming fibrin fibers (Fig. 1).

Calcium ions are key co-factors in enzymatic transformation of fibrinogen into fibrin. At this stage fibrin is dissolved in 5M urea, therefore it was called soluble fibrin.

At the same time thrombin activates factor XIII which in the presence of calcium ions (Ca^{2+}) forms lateral covalent bindings between D-domains of the fibrin monomers. Lateral aggregation of fibrin fibres leads to their thickening and enhancement of their resilient and non-rigid properties [12]. Fibrin's general mechanical properties are determined by their structural peculiarities at the level of molecules, individual fibers, as well as branching of the fibers in the fibrin network [13, 14]. Introducing factor XIII in sufficient concentration enables to obtain fibrin with good mechanical properties [15, 16]. High factor XIII concentration also catalyses cross-linking of cell adhesion proteins which enhances the adhesion properties of fibrin [17].

WAYS OF OBTAINING FIBRINOGEN PRECIPITATE AND FIBRIN GEL

Autologous fibrinogen precipitate is obtained from blood plasma by means of physical and chemical methods. Cryoprecipitation is one of the physical precipitation methods and has been described in many sources. However protocols vary largely by freezing time and temperature, as well as melting temperature and time and the number of freezing-melting cycles. Chemical methods include primarily precipitation by means of ammonium sulfate and ethanol; their combination is also used [18, 19]. Preferable precipitation methods (ethanol, ammonium sulfate and cryoprecipitation) vary according to data from various sources as the criteria of evaluation are also different. In some cases the ethanol method is preferable as there is a higher fibrinogen concentration in the precipitate and the time required to obtain it is less [20]. In other cases cryoprecipitation is called a leader as in case of sufficiently high fibrinogen concentration the

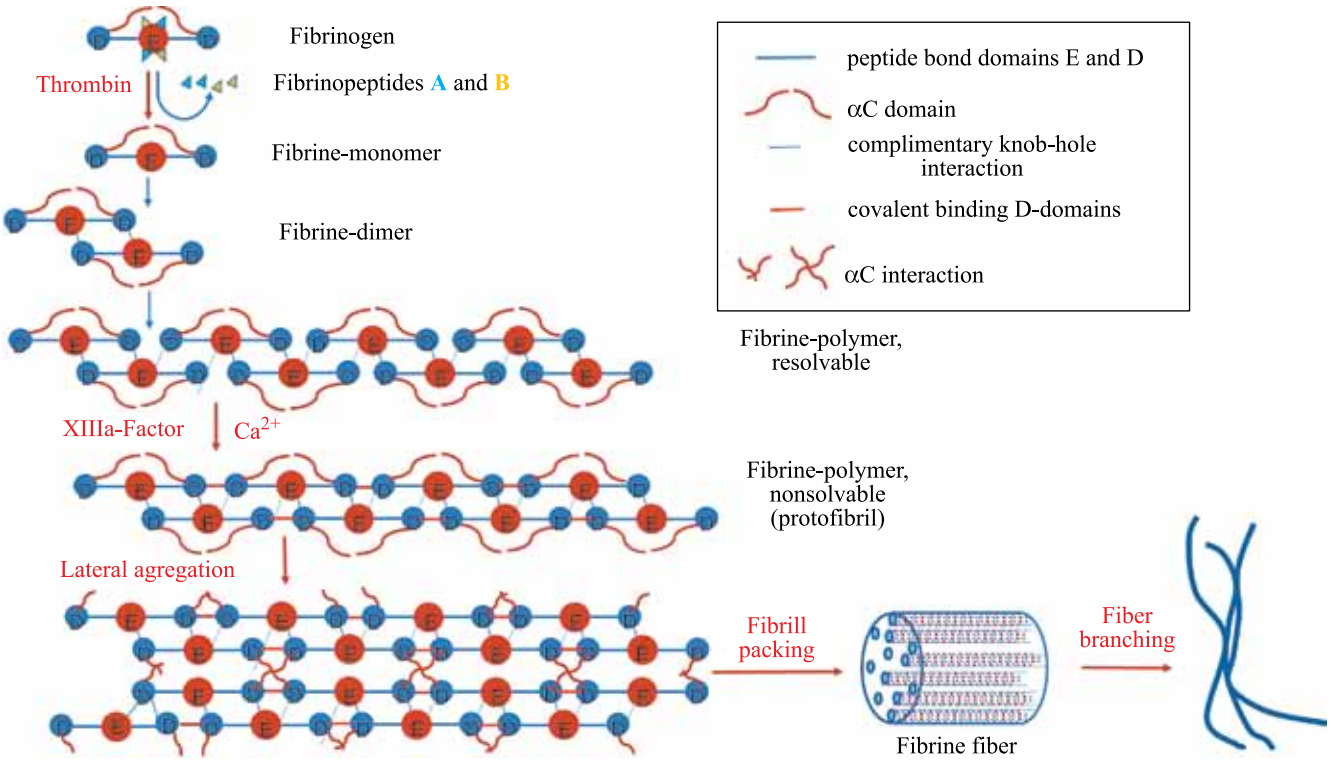

Fig. 1. Schematic diagram of the fibrin polymerization. Thrombin cleaves fibrinopeptides A and B from fibrinogen, after which the complementary regions on domains E and Dopen for knob-hole interaction. At this stage, the oligomers lengthen and form two-stranded soluble fibrin polymers. XIIIa factor in the presence of calcium ions covalently cross-links the D domains of neighboring fibrin monomers. Factor XIIIa crosslinks or ligates γ-chains more rapidly than α-chains. At this stage, the fibrin polymer becomes insoluble and obtain mechanical strength. Further, two-stranded fibrin molecules (protofibrils) laterally aggregate to make fibers, the process is enhanced by αC-interactions of D-domains. Elongation and thickening of fibrin fibers occurs at the same time with branching, as a result a three-dimensional fibrin network is formed

mechanical properties of the obtained fibrin are higher [21], which is related to partial protein denaturation during addition of ethanol or ammonium sulfate with subsequent formation of incomplete connections between fibrin monomers and fibrin clot dyshesion. Therefore different technologies for obtaining fibrinogen precipitate are used depending on the final goal.

In the majority of the studies fibrin gel is obtained from fibrin precipitate by means of introducing autologous or commercial thrombin and $CaCl_2$ [22, 23]. Tomomi Hasegawa et al. have developed and presented a non-thrombin technology of obtaining fibrin from fibrinogen [24]. In this case a polymerizing mixture is used which has low thrombogenicity as compared to thrombin, and during comparison with commercial thrombin does not activate an immune response [25].

NATURAL AND CONTROLLED FIBRIN BIODEGRADATION

Fibrin gels possess the advantage of a fully biodegradable polymer with a possibility of regulating the speed of degradation. In a normal biological environment fibrin fibers are degraded by means of fibrinolysis. In the process of fibrinolysis a non-active plasminogen, influenced by external (tissue plasminogen activator (tPA) and urokinase plasminogen activator (uPA)) and internal (XIIa factor, kallikrein) enzymatic activation is transformed into plasmin, a proteolytically active enzyme [26] (Fig. 2).

Plasmin asymmetrically cleaves fibrin into separate fragments: 'early' large molecule fibribolysis products – fragments X and Y, and 'late' fragments D and E. Fibrinolysis facilitates fibrin gel degradation with formation of non-toxic products, however the internal instability of fibrin is viewed as the main difficulty regarding its use as the main frame material or coating for TE constructions [27]. Apart from plasmin, various proteases take part in fibrin degradation, in particular an important role in this process belongs to membrane type matrix metalloproteinases (MT-MMP) [28].

Success in regulating the speed of VTE implantation is directly related to the possibility of regulating the speed of fibrinolysis. Under natural conditions fibrin decomposes within several days or weeks, the speed of fibrinolysis depends on fibrin structure, cross-linking features and protease inhibitor content [29]. Long term stability and mechanical sustainability of the matrix play an important role for the cells which require certain time and sufficient frame durability in order to remodel the matrix. Fibrin matrixes are gradually substituted by mature collagen that is synthesized by cells which have populated the full thickness and surface of the matrix (fibroblasts, EC/EPC and SMC) [27, 30].

In order to control the process of fibrin degradation two approaches are applied: additional transverse cross-linking of the fibrin fibers and use of fibrinolysis inhibitors [31]. Additional transverse cross-linking of the fibrin fibers is achieved by means of XIII factor which, apart from lateral covalent binding between fibrin γ- and α-chains, achieves transverse cross-linking with the fibrin of such molecules as α2-antiplasmin, TAFI (thrombin activated fibrinolysis inhibitor), PAI-2 (type 2 plasminogen activation inhibitor) which stabilizes the fi-

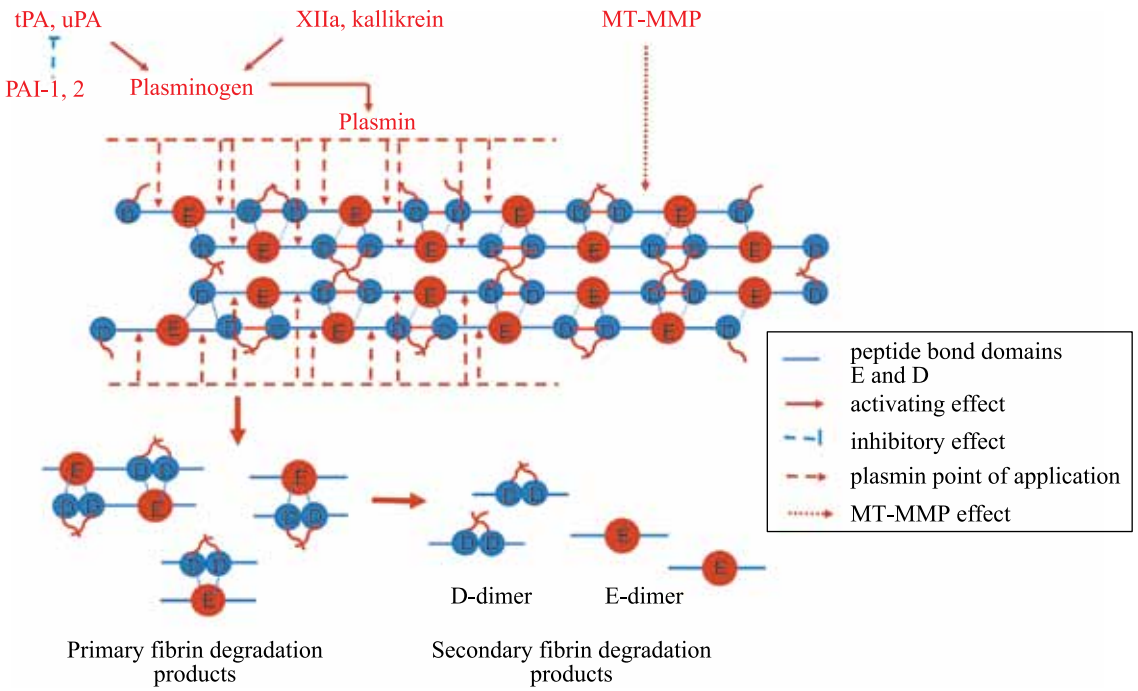

Fig. 2. Schematic diagram of fibrinolysis. MT-MMP – membrane-type matrix metalloproteinases; tPA – tissue plasminogen activator; uPA – urokinase plasminogen activator; PAI – plasminogen activator inhibitor; D – D-domain; E – E-domain

brin network and increases the resistance of cross-linked fibrin to fibrinolysis [32, 33].

In the course of developing fibrin coatings and frames on the basis of fibrin aprotinin is usually used as a proteolytic inhibitor and ε-aminocaproic acid (EACA) as a fibrinolytic inhibitor. The issue regarding the influence of these inhibitors on cell vital, proliferative and functional activity is extremely important as it determines the effectiveness of collagen synthesis and adequate tissue formation. Unfortunately there is no decisive answer to this question. There is evidence both of positive influence of the inhibitors on the vital activity of the cells [34] and of unfavourable results [35]. A study by Grassl et al. tested a wide range of EACA concentrations and absence of impact on collagen synthesis has been shown for SMC colonized on the fibrin tubular frame [35]. Opposite results have been obtained by Mol et al. [30] in whose study use of EACA inhibited ICM formation but did not hinder myofibroblast (MFB) proliferation in a human vein. These conclusions were based on a histological evaluation of tissue constructions after cultivating samples in static conditions for 2 to 6 weeks. Collagen fibers in the samples with EACA have not been detected throughout the cultivation period. Researchers believe that the inhibiting effect of EACA on collagen fiber formation is due to the fact that EACA is a synthetic analog of lysine and may play the role of competitive residue which blocks covalent cross-linking of the collagen molecules.

Nor does there exist a common opinion among scientists regarding the impact of aprotinin on cell vital activity. Both increased MFB collagen synthesis and tissue development potentiation have been described after increased aprotinin concentration [34], as well as the absence of its influence on the formation of ICM by the cells, in particular on type IV collagen and laminin synthesis [36]. In the opinion of Mühleder et al. [36], aprotinin-induced fibrin stabilization leads to a disruption in angiogenesis and formation of tubular 3D structures. The authors relate the mechanism of this effect to fibrinolysis inhibition. Nevertheless, no direct impact of aprotinin on the angiogenic properties of EC, as in the case of cultivating cells without fibrin the presence of aprotinin in the growth medium facilitates angiogenesis [37]. The impact of various aprotinin concentrations on the vasoreactive (contractility/dilatation) and mechanical properties of fibrin-based small diameter TE vascular prostheses has been described [38]. According to Swartz et al. aprotinin increases the cell colonization density and the physico-mechanical properties of TE vessels, but only owing to preserving the fibrin matrix structure. A diverse impact of aprotinin on vasoreactivity in VTE has been noted. On the one hand, it increases the capability of vasodilatation in VTE, but at the same time it decreases the receptor-mediated and non-receptor vasoconstriction [38].

Such diverse opinions regarding the impact of fibrinolysis inhibitors on fibrin properties and cell behaviour do not enable to form unanimous conclusions and require further comprehensive investigation.

FIBRIN AS A CELL CARRIER, OPPORTUNITIES FOR REGULATION OF ITS PROPERTIES

An important issue in VTE is the development of a 3D frame imitating the ICM which would facilitate cell adhesion, proliferation, migration and adequate tissue development. Fibrin imitates the ICM and not only provides physical support to the cells but also acts as a biomimetic niche, inducing biochemical and biophysical signals which regulate cellular behaviour. A 3D fibrin matrix has a nanometric fibrous structure which includes microporosity and macroporosity. The pores provide a supply of nutrients to the cells as well as migration and colonization opportunity [39]. It is known that fibrin supports expansion, migration and proliferation of various cell types, including EC [40], EPC [39], fibroblasts [41], MFB [11], mesenchymal stem cells (MSS0 [42]. The structure and physico-mechanical properties of the fibrin gel depend on fibrinogen and thrombin concentration [39, 43]. In case of low activity or concentration of thrombin there is no full separation of A and B peptides from the fibrinogen, correspondingly adequate fibrin fibers are not formed and polymerization time is increased which impacts the matrix durability significantly. In case of sufficient concentration or activity of thrombin the size of the pores and permeability of the fibrin carrier depends on the concentration of fibrinogen. Thus, increasing fibrinogen concentration within the range of 5 to 20 mg/ml decreases the size of the pores and the permeability of the matrix but increases its durability and stiffness [39, 43]. However too small pores prevent cell migration into the matrix thickness and have a negative influence on their growth and survival capability due to decreased permeability and nutrient perfusion. For the EC located on the surface of the matrix changes of the pore size do not have such a significant influence on their vital activity. Therefore depending on the goals and purposes the optimal concentration of fibrinogen should be selected individually.

Kurniawan et al. have shown that the buffer which was previously considered inert has a significant influence on fibrin self-assembly and gel permeability [44]. Buffer agents which control the pH of the media do not impact initial assembly of the fibrin monomers into protofibrils, however they hinder greatly further lateral protofibril association into thicker fibrin fibers. Buffer-mediated interruption of protofibril binding leads to a noticeable decrease in the fibrin network permeability, but does not have any significant effect on the elasticity modulus [44].

Apart from fibrinogen concentration, fibrin and pH of the buffer another factor that influences the structural

properties of fibrin is the concentration of calcium ions (Ca^{2+}). Fibrin gels with fibrinogen end point concentration 25 mg/ml and above, Ca^{2+} concentration 20 mM and pH between 6.8 and 9 possess transparency and stability properties [27]. When fibrin components are used outside these ranges a cloudy gel is formed which fully degrades within several weeks.

An optimal fibrinogen/thrombin ratio enables to obtain fibrin which supports EPC growth and their differentiation towards an endothelial phenotype with high angiogenic properties [39]. An advantage of the fibrin carrier has been noted in comparison with fibronectin. It has been shown that EPC viability, differentiation and angiogenic properties with fibrin are higher as compared to fibronectin. Besides, fibrin can support a larger number of cells functionally active for a longer time [39].

Cell distribution and proliferation on the matrixes is an important aspect for effective tissue formation. It is known that high homogeneity of cell distribution determines high ICM protein synthesis [30]. Therefore hierarchy and uniformity in cell distribution throughout the scaffold volume are of crucial significance. The work performed by T. Aper et al. showed [11] that human EC and MFB cultures, when colonized onto a composite matrix consisting of fibrin and a polyglactin frame, formed a hierarchic structure with a uniform EC monolayer on the surface and the MFB penetrating into the thickness of the frame correspondingly to the structure of the native vessel. Migration of the cells while retaining their viability, according to data presented by the authors, amounts to 519 ± 27 micron. In deeper fibrin layers no live cells have been discovered, which is related to insufficient oxygen and nutrient supply. Other research found 3D distribution of colonized fibroblasts in fibrin within a depth up to 3 mm [30, 45]. The average thickness of a human coronary artery varies between 390 to 1300 micron; therefore, maximal depth providing for MFB viability in a fibrin matrix will enable to create a small diameter vessel. Thus, fibrin gel as a cell carrier, depending on the cell type, ensures their hierarchic and uniform distribution, facilitates free migration and proliferation on the surface and within the matrix. This will enable to quickly and effectively colonize fibrin and form a compact formula. Also, cellular synthesis of ICM proteins within the matrix prevents washout of the soluble components and provides for ICM maturation in a short timeframe.

Good elasticity of fibrin gel is another important aspect of its effectiveness as a cell carrier. Elasticity determines the functional characteristics of the cells, influences the course of angiogenesis, regulates cell migration and traction force [46]. Fibrin possesses both elastic and viscous properties which are directly related to displacement deformation. Displacement deformation, in its turn, induces the launching of the signal mechanotransduction complex which regulates the adaptation of the cells to their physical environment. Fibrin fibers are exceptionally distensible and elastic [47], which sets fibrin advantageously apart from other protein fibers [48].

FIBRIN AS A DIRECT ANGIOGENESIS REGULATOR

In the process of wound healing the fibrin clot not only limits blood loss but also ensures release of a number of factors which stimulate new blood vessel formation. The classic model of wound healing accepts the active role of cells which influence the behaviour of other cells by means of signal transduction, however at the same time a passive role of a basis is assigned to the ICM [49, 50]. It has currently been proved that the fibrin matrix takes active participation in the regulation of angiogenesis, colonization and cell invasion [51].

The matrix structure contains not only cell integrin binding sites but also determines the speed and degree of the proteolytic degradation of the matrix induced directly by the cells themselves [52]. The factor regulating and controlling EC fibrin colonization and invasion is fibrinolysis which in turn is determined by metalloproteinase and plasmin system activity [51]. Hemostasis and angiogenesis are two interrelated physiological processes which act in a balanced and concordant way in order to restore microcirculation after vascular disruption [50]. Directly after the injury it is important to prevent excessive bleeding, and effective coagulation is needed for this. Starting angiogenesis at this stage is unproductive and premature, as newly formed vessels are delicate and unstable [53]. Therefore thrombogenesis and angiogenesis processes are strictly controlled, and angiogenesis is launched only after hemostasis has been successfully completed. Proof of direct impact of fibrin on angiogenesis is presented in a paper by Hadjipanayi et al. [54]. Fibrin binds many pro- and antiangiogenic factors which are released after coagulation (transforming growth factor β (TGF-β), vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), platelet factor 4 (PF4), thrombospondin 1 (TSP1)) [55], and it also takes part in controlled release of these factors and angiogenesis regulation. The fine regulation mechanism is closely related to fibrinolysis and hemostasis processes. At early stages the fibrin clot performs a hemostatic function and initiates controlled release of angiogenic inhibitors, at the same time it facilitates EC attraction and migration by means of chemotactic factors. At the stage of a formed fibrin clot and activation of fibrinolytic processes fibrin gradually increases the release of proangiogenic factors and decreases the release of antiangiogenic ones, thus creating conditions for effective angiogenesis.

FIBRIN AS A MEANS OF GROWTH FACTOR DELIVERY

Fibrin is an independent cell behaviour regulator. In the course of morphogenesis and tissue healing the release of growth factors (GF) from the ICM plays a key role for the attraction of cells, regulation of their distribution zones and signal transfer [56, 57]. In this process GF release should be carefully balanced in order to achieve proper tissue formation.

Fibrinogen and fibrin are able to bind and retain GFs from different groups (PDGF, VEGF, FGF, TGF-β) via a heparin-binding domain nonspecifically with high affinity [55, 58]. GFs which are not bound to fibrinogen/fibrin are quickly eluted from the fibrin matrix, while those which are bound are retained for a prolonged period and released slowly. Nonspecific high affinity of fibrin to GF plays an important role in damaged tissue restoration, because fibrin acts as a reservoir for the delivery of growth factors, facilitating their reparation and healing.

Slowing down of the GF release process may be achieved by means of pressing fibrin, which at the same time increases frame density and improves the physicomechanical properties of the construction [53]. Another way of increasing GF content in fibrin is related to the use of plasma rich in platelets while manufacturing the matrix. Platelets contain a large number of GFs, such as PDGF, TGF-b1 and TGF-b2, FGF, VEGF and insulin-like growth factor (IGF), which stimulate cell proliferation, matrix remodeling and angiogenesis [54]. Use of platelet concentrate during fibrin matrix formation is an interesting option for tissue healing facilitation [55], however it is not suitable for creating vascular prostheses as it increases the thrombogenicity of the construction. Apart from the physiological ability of fibrin to bind and release GF, the fibrin gel/matrix enables to incorporate growth factors, bioactive peptides and proteins [56] which opens additional opportunities for focused differentiation and influence on the vital activity of colonized cells.

THE VASOREACTIVITY OF FIBRIN-BASED VTE

An ideal VTE graft should adapt to changing conditions of the blood flow, i.e. manifest vasoreactivity. The main elements in vasoreactivity are the smooth muscle cells which are able to respond to contractile and dilatation stimuli. Owing to contractile properties and proper radial location of vascular smooth muscle cells conditions are created not only to increase frame strength and stability but also to achieve vasoreactivity for the whole construction. Swartz et al. have been studying the properties of fibrin-based small diamteter VTE colonized with SMC and EC [32]. The constriction and relaxation ability of VTE have been evaluated after exposure to various vasoactive substances on the circular segments of the construction. The VTE achieved significant mechanical performance and vasoreactivity already after 2 weeks of maturation under static conditions. After implantation the process of remodeling was continued with an increase in mechanical performance and vascular reactivity.

FIBRIN INCREASES CELL RESISTANCE TO SHEAR STRESS

EC play a decisive role in vascular biology and perform various functions: serve as a selective permeability barrier, take part in thrombogenesis regulation and thrombolysis, platelet adhesion, vascular tone modulation, immune and inflammatory response regulation, mechanotransduction. EC loss induces local activation of a pathophysiological cascade leading to neointimal hyperplasia [59]. Therefore formation and support of a functional athrombogenic endothelial monolayer is a key requirement in VTE development.

Under physiological conditions EC are subject to complex mechanical exposure which includes shear stress, pressure and radial distention. It is known that over 70% of the EC colonized on a synthetic frame are washed off within the first 20 minutes after implantation and this process is intensified in case the shear stress level is increased [60]. Covering the construction with adhesive proteins enables the EC to withstand the flow. The adhesive proteins used for this include fibronectin, collagen, gelatin, whole ICM and fibrin. The most comprehensive comparative characteristic of cell retention on the surface of a vascular prosthesis covered with various types of protein is presented in the work by Chlupáč et al. [23]. The researchers studied retention properties for human EC on commercial prostheses made from PET with type I collagen and similar prostheses additionally coated with laminin, fibrin and fibrin/fibronectin. The effectiveness of cell colonization after 4 hours of interlaminary cultivation under rotation conditions amounted to 22–30% of the initially introduced amount and did not vary significantly in the prostheses with or without coating. After 3 days of maturation in static conditions the colonized prostheses were placed into a bioreactor. A significant and progressing with time loss of cells was observed on the commercial and laminin-coated prostheses. Under the same conditions a less significant loss of cells was taking place from the surface of prostheses coated with fibrin and fibrin/fibronectin, and by 120 min even an increase in their number was noted. The researchers relate the increase in the number of EC on a surface coated with fibrin or fibrin/fibronectin to the beginning of cell proliferation.

Adhesion to fibronectin and fibrin involves integrin receptors αvβ3 and α5β1 [61], which play a decisive role in adapting EC to hemodynamic efforts; their activation launches intercellular signal transfer and a change in the expression of genes which activate angiogenesis and proliferation [62]. The abovenamed receptors are not involved in EC adhesion to collagen and laminin [61], which emphasizes the advantage of EC interaction with

fibrin in order to form the desired cellular responses. The ability of fibrin to retain EC under shear stress conditions does not depend on the type of polymer basis (PET, PTFE, ePTFE) nor on the type of fibrin use (as a polymer coating or as an independent frame) [63]. Totally fibrin scaffolds also enable to obtain high density endothelization and support the monolayer under physiologically significant shear stress conditions [63].

Unfortunately coating of the graft with adhesive proteins (ICM, plasma or fibronectin) apart from improving EC adhesion also facilitates platelet adhesion and aggregation and increases the risk of thrombogenesis. As compared to whole ICM and gelatin, fibrin demonstrates lower platelet activation and adhesion on the surface and consequently lower thrombogenesis [64]. Thus, fibrin is the material of choice in supporting VTE endothelization under pulsing blood flow conditions.

FIBRIN MOLDING

Fibrin polymerization takes place during a certain period of time which has its positive aspects in the creation of TE constructions. There exist various technologies of scaffold manufacturing: mold casting in order to create a 3D porous structure and frame saturation for modification or for the creation of a fibrin coating.

In the first case fibrin components are poured into the desired mold, they are allowed to polymerize and then the ready construction is extracted from the form. Due to initial fluidity of the component mixture during fibrin molding it is possible to obtain precise and complex forms, for example valves and complex vessels with side branches [65]. The fluidity property enables to saturate different types of molds which creates a stable fibrin layer on the surface and imparts new desirable properties to the construction [23]. Moreover, introducing cell culture into the suspension or solution before mixing and polymerization facilitates uniform cell distribution in the inundated matrixes. Owing to the properties of fibrin which are extremely favourable for cell colonization and further remodeling in the body, technologies for manufacturing fibrin small diameter vessel prostheses by molding are being actively developed [15].

However using exclusively fibrin as the basis for VET encounters the main problem – insufficient mechanical performance of the fibrin gels in order to withstand physiological dynamic stress *in vivo*.

LOW MECHANICAL PERFORMANCE OF FIBRIN, OPTIONS FOR PROBLEM SOLUTION

Commonly used techniques aimed at improving mechanical performance of fibrin gels include two areas: improving the performance (strength) properties of the fibrin itself and creating a frame or hybridization with polymers/tissues.

Impacting on fibrin in order to improve its performance and durability characteristics includes several options: increased fibrinogen and thrombin concentration (described above); cross-linking of the fibrin fibres by XIIIa factor; densification of the fibrin structure by means of centrifugal force or compression; colonizing fibrin with cells during subsequent maturation in a bioreactor or in static mode. In studies aimed at VTE creation both separate methods are used as well as a combination of several methods [15, 16].

Cross-linking with XIIIa factor not only prevents fibrinolysis but also improves the performance characteristics of the fibrin clot. XIIIa factor enables to improve the resilience modulus for the fibrin fibers by over 8 times, and the ultimate resistance by 4.5 times [12, 16].

Improvement of the physicomechanical properties of the fibrin clot is to a certain extent facilitated by mechanical stimulus during polymerization. After being subject to continuously fluctuating forces of sufficient intensity a more rigid and durable fibrin matrix is formed as compared to similar matrixes which have not experienced mechanical pressure [66]. Thomas Aper et al. have presented a technology of high speed centrifugal molding, which enabled to sufficiently improve biomechanical stability of the fibrin tubular frames when the speed of rotation for the press mold was increased from 1000 to 1500 rpm [15]. The main principle of centrifugal molding is related to removing excess fluid which amounts to over 80% of the fibrin matrix volume; the densification of the fibrin clot induced by this facilitates cross-linking of the fibrin fibers. It has been noted that isolated centrifugal molding is less effective than a combination of similar parameters of centrifugal force and XIIIa factor cross-linking. Moreover, introducing EC and SMC into the fibrin content along with adding XIIIa factor additionally improves the performance of the tubular fibrin construction [15].

Colonizing the fibrin construction with fibroblasts, SMC and EC facilitates strengthening of the wall by means of forming cell interactions and ICM protein synthesis. However in order to improve the physicomechanical properties after *in vitro* cell colonization the construction should undergo a period of maturation under static or dynamic conditions. Pulsing flow conditions speed up and increase the effectiveness of fibrin construction maturation. Conditioning of the fibrin frame colonized with fibroblasts under conditions of a pulsing bioreactor improve the performance of the construction within 7 weeks to the parameters of the native artery [67].

Insufficient durability and rigidity of the fibrin matrixes is closely related to the problem of fibrin shrinkage [65]. The impact of the pulsing flow on the cell-colonized fibrin construction decreases the percentage of fibrin shrinkage [68]. A pulsing flow stimulates expression of cytoskeletal proteins, aligns the colonized cells along the direction of the flow, and also significantly increases ICM protein synthesis and accumulation; this improves durability and resiliency properties of the matrix, acti-

vates remodeling and creates a resistant frame which prevents shrinkage [68].

Forming a strengthening fibrin-based polymer frame or reinforcing net not only improves durability characteristics but also decreases the period of maturation for the fibrin construction. Thus, creation of a quickly degrading frame made of polyglycolic acid with poly-4-hydroxybutyrate on a fibrin basis colonized by MFB decreased the preparation time to 4 weeks [69]. After 4 weeks of *in vitro* dynamic deformation the mechanic properties of VTE obtained indicators comparable to those of the internal mammary artery (burst pressure level 903 ± 123 mm Hg). A shorter period of preparation is described in the case of strengthening the wall of a MFB-colonized autologous fibrin vascular prosthesis by a polyvinildenfluoride (PVDF) reinforcing net [70]. During 2 weeks while the construction was placed into bioreactor conditions with physiological flow velocity and pressure gradient parameters it was possible to increase the average suture retention durability to 6.3 N, and the tensile breaking strength to 236 mm Hg. At the same time the fibrin gel matrix showed good tissue development and high concordance with native vessels.

Reinforced small diameter fibrin VTE show very promising results after implantation: high patency, no calcifications or aneurysms, signs of active wall remodeling (presence of SMC and mature autologous ICM proteins) and EC monolayer formation on the inner surface of the graft [71].

The electrospinning method is gaining increasing popularity in the making of vascular prostheses. It enables to form nanosize fibers and to create a porous frame structure. An option has been suggested to create a fibrin frame strengthened by an ultrathin fibrous cover made of poly-ε-caprolactone (PCL) with the use of electrospinning, which enables to get a small diameter vascular prosthesis ready for implantation within 1 week [72]. A week after implantation to mice these prostheses obtained performance properties similar to those to be found in native arteries. Fibrin-mediated cell remodeling, stable intima, massive matrix accretion with organized collagen layers and elastin fibers were formed already at week 4 after implantation. Grafts showed high patency, low thrombogenicity and an inclination to calcification. This technology has a promising development prospect, however further research is required using larger animals and increasing the implantation period.

The concept of hybrid scaffolds which combine the advantages of natural and synthetic materials is very attractive and is believed to be effective. Creating coatings on the surface of polymers and decellularized tissues are meant to set up cell adhesion sites and increase biocompatibility; in this context a lot of attention is devoted to fibrin coating. In this technology form molding and the main mechanical stress is borne by the polymer frame or the decellularized tissue, and the fibrin coating creates a biocompatible layer on the surface which makes cellular invasion and growth easier. Good penetration power of the fibrin gel, good linkage with textured or porous surfaces after polymerization enable to use fibrin as a coating or for the saturation of frames made from various polymer materials or xenogenic tissue [23, 71, 73]. As a polymer basis for biomimetic hybrid frames with biological properties of fibrin the following are used: porous PCL [74], polylactide [71], PET [23], PTFE/dacron [75]. In order to retain mechanical properties and improve biocompatibility biological composite frames are developed on the basis of decellularized arteries covered by fibrin gel [73]. It has been suggested to manufacture a hybrid frame by applying aerosol fibrin gel on the inner and outer surfaces of decellularized arteries with successful cell colonization (MFB and EC). In this case the decellularized fibers of the carotid artery are closely interwoven with fibrin gel fibers and the layers are firmly bound to each other. The obtained hybrid frames / vascular grafts and native arteries demonstrate similar physicomechanical properties [73].

Thus densified and reinforced (frame-mounted) fibrin, as well as hybrid frame options can be potentially bases for creating effective small diameter vascular prostheses.

OPPORTUNITIES FOR CREATING FIBRIN-BASED AUTOLOGOUS GRAFTS

One of the main goals in VTE is the creation of a fully autologous prosthesis for small diameter vessels. The discovery of vascular EPC enables to obtain pure cultures of autologous EC even from patients with CVDs. EPCs can be obtained from the patient's own peripheral blood. An increase in the number of EPCs in response to a mechanic vascular damage, hypoxia and ischemia expand opportunities for EC recovery from blood which presents the most available, continuous and sustainable source of autologous endothelial colony forming cells (ECFCs) [76, 77]. Besides, it is possible to isolate colony-forming late outgrowth smooth muscle cells (LOSMC) from blood [78, 79], which are necessary for colonization of the vascular prosthesis wall.

Aper et al. have been successful in obtaining a fully autologous VTE prosthesis by having united all the three components: fibrin frame, ECFC and LOSMC [15]. In order to get a highly compact fibrin matrix with satisfactory physicomechanic properties cross-linking by XIIIa factor, high speed centrifugal molding, colonization with ECFC and LOSMC which had been isolated from peripheral blood were used. The construction was implanted to sheep for 1 and 6 months. As a result of active remodeling of the prosthesis wall after 6 months a structure similar to the native artery was formed. Fibrin was replaced by newly synthesized ICM proteins, cell and capillary ingrowth from surrounding tissue into the implanted segments was noted; as a result the biomechanical properties began to correspond to the properties of

the native artery. Despite evident success the durability properties of the prostheses before implantation did not quite correspond to those of the native artery, and one of the sheep died from prosthesis rupture in the process of the operation. A possible solution could be additional introduction of a maturation stage for the colonized fibrin construction in the conditions of a bioreactor, which would somewhat increase the time for preparing the construction before implantation. Nevertheless the results are encouraging and there is hope that functional, fully autologous vascular prostheses may be obtained from available sources of the patient's own cells and tissues.

CONCLUSION

Fibrin is a promising material in VTE. It has a great potential for molding which enables to obtain complex 3D shapes. A fibrin frame possesses unique tools for cell adhesion, migration and retention under shear stress; it is able to control angiogenesis, to accumulate and gradually release growth factors. The opportunity to control biodegradation, as well as the morphobiological properties of fibrin, enable to select and save the required characteristics. The listed advantages of fibrin in combination with availability of the source and lack of immune response after implantation make it an ideal frame for developing VTE grafts. Fibrin gel possesses an inner potential, largely exceeding other matrix materials with the exception of mechanical durability. Throughout the world search for ways of improving mechanical durability of fibrin matrixes is going on; in this case hybrid scaffold technology or fibrin reinforcement may become one of the options for solving this problem. Moreover, creation of a fully autologous fibrin-based TE vascular prosthesis may be possible.

The authors declare no conflict of interest.

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CLINICAL, IMMUNOLOGICAL AND ETHICAL ASPECTS OF SELECTING A RECIPIENT FOR CADAVER KIDNEY TRANSPLANTATION

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The decision to choose a particular patient for kidney transplantation is made through two consecutive decisions: decision to include the patient on the waiting list and decision to select a patient competitively among several candidates for transplant. Both decisions are taken amidst many competing priorities and require a multidisciplinary approach. This paper provides comparative analysis of the principles of maintaining a waitlist and selecting a donor–recipient pair in Russia, Europe (Eurotransplant) and the USA (UNOS). *Donor–recipient pair is selected* based on the traditional hierarchical scheme of decision rules. Unlike Eurotransplant and UNOS, there are no uniform standards in Russia for assessing the quality of a donor organ. The widespread and largely vague "old for old" principle should be harmoniously fitted into the general outline of donor kidney distribution. The second difference in the national distribution system of donor kidneys is the choice in favor of a candidate with a lesser degree of sensitization. With high frequency of positive cross-test, this principle, in a synergistic manner, greatly reduces the availability of transplantation for highly sensitized candidates. The quality of donor organ and unconditional priority on highly sensitized candidates are the conceptual fundamental principles of organ distribution in the US and Europe. Under donor kidney shortage, selecting a recipient is always competitive. The choice of a candidate can be based on a patient-oriented approach (a choice in favor of the candidate whose transplantation will most likely reduce the risk of death; for example, an "emergency" waiting list) or an alternative – a utilitarian approach (choosing the candidate with the longest predictable life expectancy). However, radical commitment to one of these approaches inevitably reduces availability of kidney transplantation for a specific category of patients. For a justified choice of recipient, it is necessary to correlate such factors as comorbidity, waiting time, age, histocompatibility and quality of donor kidney. This would achieve a shaky balance between utilitarian approach and patient-oriented approach. The principles of creating a waiting list and a system for efficient distribution of donor organs practiced by foreign organizations cannot be simply copied and reproduced in Russia. It is necessary to adapt and validate such principles for the local patient population. The objective difficulties of such an analysis dictate the need to address it on a national scale. This would ensure equitable distribution of donor organs to all patients in need and obtain the best transplant results. Moreover, this would make it possible to achieve the full potential of donor organs. *Conclusions*. The situation in transplantological and nephrological care in Russia is gradually changing. This determines the need to adapt and standardize approaches to allocation of cadaveric donor kidneys in order to ensure equal access to transplantation for different patients and fullest realization of their potential. Removing organ distribution from the area of responsibility of local coordination councils, introducing a unified policy for distribution of donor organs and choosing a specific recipient will reduce the subjectivity of decisions and, possibly, improve transplantation results.

Keywords: kidney transplantation, waiting list, donor, recipient, comorbidity, histocompatibility, organ distribution.

According to the Declaration of Istanbul [1] organ transplantation, being one of the miracles of the twentieth century and a radiant symbol of human solidarity, continues saving and improving the lives of hundreds of thousands of patients throughout the world. It is generally accepted that kidney allotransplantation (ATP) is the optimal method of renal replacement therapy (RRT). Large studies have actually confirmed that the RRT method provides full medical and social rehabilitation for the patients as well as improved quality of life [2] and, most importantly, ensures the highest survival rate among patients with chronic kidney disease (CKD) [3–5]. This is true for the overall population of patients with CKD, however a practicing physician inevitably encounters the need to use the top-down approach, i.e. to determine the optimal RRT method for the specific patient. We will not discuss the advantages and drawbacks of hemodialysis or peritoneal dialysis and will concentrate on the approaches of patient selection for kidney transplantation, devoting a special attention to

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aspects relevant for our country. As it has been truly said in the European clinical recommendations on best clinical practices in examination and follow-up for donors and recipients for donor kidney transplantations [6], "… patient care after kidney transplant requires special knowledge in such areas of medicine as nephrology, immunology, pharmacology, endocrinology, communicable diseases and cardiology". On the one hand, this indicates the need of a multidisciplinary approach to management of candidates for transplantation and kidney transplant recipients; on the other hand – a potential possibility of a large number of contradicting and competing priorities present.

The choice in favour of ATP is implemented in the form of two subsequent decisions: including the patient into the waiting list and selection of the donor-recipient pair by means of determining a particular candidate for transplantation. We believe that the second decision is a harder one to make.

In Russia in routine clinical practice for kidney transplant, apart from the blood group, incongruity by three pairs of human leucocyte antigens (HLA) – first (HLA-A, -B) and second (HLA-DR) classes – are taken into account, namely, the number of the donor's antigens which are absent in the recipient [7] (with priority importance assigned to a minimum number of incongruities/mismatches by DR locus antigens). As practically each organ transplant center in our country has a small self-contained waiting list, opportunities for choosing an ideal candidate, taking into account tissue compatibility, are significantly limited. As a result in the majority of the cases the physician gets a list of patients who are equal from the point of view of tissue compatibility (equal number of HLA incongruities). There is no doubt that during the choice of a candidate for transplantation the physician is guided exclusively by humanistic intentions. At the same time, this choice is not quite as unambiguous as it might seem at first sight, and inevitably creates an ethical dilemma.

By the end of 2016 5050 patients have been included into the waiting list for kidney transplantation. During the year 2017 1175 kidney transplantations have been performed and 1925 patients have been added to the waiting list. Thus, taking into account those patients who had died or had been excluded for various reasons, by the end of 2017 the list contained already 5531 patients. It is clear that under conditions of such a shortage the choice of recipient is always competitive.

The choice of a recipient may be based on one of the two approaches. The first approach is patient-oriented. The main principle is decreasing the risk of death *at the moment of transplantation*. In order to implement this project the risk of death after ATP is related to the risk of death in case dialysis treatment is continued. The patient who has a higher *relative* risk of death is chosen as a recipient. According to this principle, for example, transplantation is performed for a patient who has been on the waiting list for a longer period of time. At the same time, as it has been shown [9], increasing the waiting time leads to the deterioration of the comorbid background. Apart from that, in accordance with this principle a so-called 'emergency waiting list' is formed (a typical example may be patients with exhausted possibilities of vascular access forming and impossibility of RRT conversion to peritoneal dialysis). In case of a favourable transplantation result the patient's lifespan may be quite long, while continuing hemodialysis treatment is related to a high risk of rapid death. Thus, transplantation enables to decrease manifold the risk of death, while a stable patient with a good functional arteriovenous fistula will gain significantly less benefit from ATP.

The patient-oriented approach facilitates a decrease in mortality for patients receiving dialysis (naturally among patients in the waiting list) as increased risk of death increases proportionally the likelihood of transplantation. However increased probability of transplantation along with increased risk of death is not monotonous: the patient may be excluded from the waiting list if the risk of transplantation exceeds the risk of death while receiving dialysis. Nevertheless, taking into account the competition for each donor organ, this approach inevitably affects also the other patients in the waiting list. As a result the average waiting time is increased for all the remaining candidates as a candidate who had been waiting for a long time will have a priority before a candidate with a shorter waiting time. This naturally facilitates a decrease in the life expectation for the other patients, as well as a decrease of the overall survival for the recipients after ATP (as this approach leads to an accumulation of a pool of recipients with lower life expectation). A positive aspect in this approach is a significant increase in the availability of transplantation for patients with burdened comorbid background. However at the same time the likelihood of transplantation is decreased in patients with a better comorbid background (this probability increases with the increase of time in the waiting list). The overall principle of this approach can be expressed by the words 'transplantation as a means of saving life'.

The other approach, an alternative one, may be described as utilitarian. According to it the preference ifs given to the candidate who has the longest predicted life expectation. This facilitates an increase in the overall survival of the recipients as well as a decrease of the overall time of waiting for transplantation. At the same time, this approach significantly decreases the probability of transplantation for patients with low predicted life expectation (as they are likely to not live long enough for transplantation or be excluded from the waiting list due to a deterioriation of the comorbid background) and may also increase the mortality among patients receiving dialysis (due to accumulation of a pool of patients in a worse condition, while patients in a better condition

will receive the transplant). The overall principle of this approach can be expressed by the words 'ensuring a maximally effective use of the donor organs from an utilitarian point of view'.

Thus, in practice a physician often faces the choice between performing transplantation for a patient with a higher risk of death, thereby somewhat worsening the prognosis for a patient with a lesser risk. Or the opposite: to give the preference with a higher predicted life expectation, therefore significantly increasing the risk of death for the patient in a worse condition. Radical commitment to either one of these alternative methods will inevitably decrease access to transplantation for a certain category of patients.

Under conditions of donor organ deficit such a dilemma will inevitably arise. It is clear that a compromise between these approaches is necessary in order to ensure a shaky balance between two ethical principles: potential benefit for the candidate's health and equity in organ distribution. Let us have a look at the experience of our international colleagues – large donor organ distribution systems such as Eurotransplant – ET (Europe) and United Network for Organ Sharing – UNOS (USA).

The system of donor organ distribution at ET is implemented by means of two programmes: Eurotransplant kidney allocation system (ETKAS) – kidneys from donors aged below 65 years old – and Eurotransplant Senior Program (ESP) – kidneys from donors aged 65+ [10]. Under the ETKAS protocol the patients are graded by blood group compatibility (according to the appropriate schemes) taking into account the following criteria: sensitization level (panel-reactive antibody – PRA), tissue compatibility (HLA-A, -B, -DR), time on the waiting list, HLA-mismatch probability and region of retrieval. This algorithm is intended primarily to ensure optimal immune compatibility of the donor (post mortem) and the recipient, at the same time it takes into account the time of waiting for transplantation.

The ESP protocol is used primarily in order to decrease conservation time and optimize the use of 'aged' donor organs. The organs are distributed sequentially at the local, regional and national level, not taking into account the donor's HLA phenotype among non-immunized recipients aged 65+ and ranged on the basis of urgency and waiting time. Immunized patients (those with previously existing anti-HLA antibodies) are included into the distribution according to the acceptable mismatch programme which permits transplantation from other groups. Transplantation through the EKTAS and ESP systems, according to ET policy, envisages transplantation exclusively when the donor's and recipient's blood groups match by the AB0 system.

Thus, the 'old for old' concept has not only been successfully implemented but also harmoniously introduced into the general framework of donor kidney distribution. This, on the one hand, increased the access to transplantation for elderly patients, and on the other hand, facilitated increasing the possibility for a young recipient to get an organ from a young donor. Short-term results after introducing the ESP protocol (in 1999) were quite promising due to significantly decreased conservation time (owing to priority distribution of the organs through this programme at the local level) [11]. Long-term results proved that the goal of the program had been achieved: the access to transplantation for elderly people has been increased, the waiting time for transplantation and the length of the conservation time have been decreased, the frequency of delayed renal graft function has been decreased. At the same time the frequency of rejection episode crises has somewhat increased and the graft survival has decreased as compared to recipients in the same age group who had received kidneys from younger donors [12]. Nevertheless survival rates among recipient were higher than survival rates among patients of the same age group from the waiting list [13, 14].

Thus, the key criteria for donor kidneys in ET are the donor's age and the candidates' sensitization. These primarily determine the choice of the protocol according to which the recipient will be selected.

In the USA the system of donor organ distribution with a common waiting list was introduced in 1977. One of the main criteria for recipient selection was the length of dialysis. An increased need in donor organs led to the need to use expanded criteria donors (ECDs). These included donors aged 60 and above, as well as donors aged 50–59 with at least one of the following criteria: serum creatinine over 1.5 mg/dl, death due to cerebrovascular causes or pre-existing arterial hypertension. Grafts received from such donors had over 70% higher risk of function loss [15]. At the same time the ratio of grafts obtained from ECDs in 2005 amounted to 17% of all post mortem donors [16]. When being included into the waiting list the patient made a choice regarding the possibility of receiving a kidney transplant from an ECD. Kidneys obtained from ECDs were distributed among patients who had agreed to participate in this programme in the following order: patients with no HLA mismatch at the national level, all other patients at the local, regional and national level taking into account the length of waiting time but not HLA compatibility [17].

The purpose here was a desire to decrease the time of waiting for a transplant. As large studies have shown, elderly patients had a low life expectancy and high risk of death with a functioning graft. At the same time younger patients with longer life expectancy after transplantation 'outlasted' the period of the graft's functioning and returned to dialysis and waiting for second-set grafting [18]. This enhanced the shortage of donor organs. A need arose to optimize the donor organ allocation system.

The 2008 American National Kidney Transplantation Concept [19], apart from HLA compatibility, was based on three main criteria: dialysis duration, panel-reactive

antibody (PRA) indicator and the life years from transplant (LYFT) ratio. The implementation of LYFT as an estimate indicator became a conceptual component of the evolution of the allocation system. LYFT is determined as the difference in life expectancy between two alternative options of the course of events, in one of which the patient will receive kidney transplant at the moment *from a specific donor* (total life expectancy with functioning graft, as well as after the loss of its function and resuming dialysis treatment), and in the other one – the patient continued dialysis treatment. Another important innovation was that while calculating this ratio the quality of life was indirectly taken into account: during calculation of life expectancy during dialysis treatment (in both options for the course of events) a reduction factor was applied (0.8) [20].

Changes also took place in the system of donor organ quality assessment. A binary classification (standard donors / donors with expanded criteria) has been replaced by a continuous scale. Donor kidneys were ranged in accordance with the kidney donor risk index (KDRI) [21] that indirectly reflected the potential of their functioning duration. This index, among other criteria, also included HLA compatibility. The allocation of donor kidneys with the highest expected functioning duration is influenced to a larger extent by the LYFT indicator (80%) and to a lesser extent (20%) by the dialysis duration as well as by PRA. The priority of the factors changes linearly along with deterioration of the donor kidney quality. Donor kidneys with the shortest expected functioning duration period are allocated only taking into account the dialysis duration and PRA [22]. Thus, candidates with the highest life expectancy received a priority in the allocation of kidneys with the highest potential survival. Patients who had been on the waiting list and receiving dialysis, on the other hand, received a priority in the allocation of kidneys with a shorter expected functioning duration. As a result transplantation availability for both patient categories has been improved.

The main arguments expressed by critics of the currently existing system of organ allocation were its complexity as well as low access to transplantation for highly sensitized patients. Also the LYFT calculation concept has been criticized. Despite the elegance of theoretical constructions and the information value of this assessment which has been proved during development, the experience of its use has shown insufficient precision of the prognosis. This has been related to a limited number of predictors that are used in the calculations [23]. For example, in the LYFT calculations the risk of cardiovascular events is not included (though such a possibility has been viewed in the course of developing the calculation method for this indicator [20]). At the same time it is known that cardiovascular diseases are the main cause of death for patients with stage 5 CKD [4, 5]. Kidney transplant recipients also have a higher risk of death from cardiovascular diseases than the general population rates [24] (though to a lesser degree than for patients receiving dialysis) [4, 25–27]. Thus, two candidates comparable by criteria included in the LYFT calculations may have significantly different prognoses.

The next stage of developing the allocation system in the USA towards a more comprehensive use of donor kidneys became the abandoning of LYFT and KDRI in favour of calculating a significantly more simple indicator – the expected post transplant survival (EPTS) rate. At the same time the quality of donor kidneys was evaluated by a new index – the kidney donor profile index (KDPI) [28, 29].

In the course of KDPI calculation the donors' age, height, weight, ethnic background are taken into account, as well as arterial hypertension and diabetes in the medical history, the cause of death, the blood serum creatinine level and hepatitis C status. In the course of EPTS calculation the recipient's age, diabetes status, previous organ transplantations and dialysis duration are taken into account.

The algorithm of recipient selection is carried out according to the traditional hierarchic scheme of decision functions. The initial link here is the donor organ quality – KDPI: on the basis of this score the kidneys are allocated to one of four categories, each of which has its own sequence of recipient choice. This system has two key principles. The first one is that the EPTS rate is calculated only for the best quality kidney allocation (for KDPI ≤20%). Thus, equity in the access to transplantation is assured.

The other key principle is an uncompromised priority for highly sensitized candidates (PRA 98–100%) and candidates with no HLA mismatches at the A-, B- и DRB1 HLA loci (this principle is observed for the allocation of kidneys with any KDPI rate) [18, 20, 30].

During kidney allocation the candidates are ranged in accordance with the number of points received for dialysis duration, PRA rate, compatibility by HLA-DRB1 locus. Additional points are allocated to children and patients who have become donors while alive. Same as in the previous system of kidney allocation, candidates for simultaneous transplantation (kidney and an extrarenal organ) have a priority.

An important feature of this system is a possibility for transplantation of cadaver kidneys to candidates from other groups with no HLA mismatches.

Implementation of the new system of donor organ distribution in 2014 has led to a small but statistically significant increase in the conservation length (from 15.8 to 16.8 hours), decrease of the average age of the recipients (from 55 to 52 years), increased correlation between the age of the donor and the recipient (from 0.35 to 0.38), decreased proportion of recipients without HLA mismatches (from 8.5 to 4.5%), decreased proportion of recipients above age 30 (from 19.4% to 15.0%). This led

to a significant increase in the proportion of recipients with PRA 100% (from 1.0% to 10.3%) [31]. At the same time the number of recipients below age 40 increased by 81.7%, and the number of recipients aged over 65 who received transplants with KDPI ≤20% decreased by 65.8% [32].

The long-term results of implementing this system are yet to be evaluated: it is not known how the survival of the recipients or of the transplants will change, as well as the proportion of recipients who die with a functioning transplant. However it is one of the most well-reasoned and balanced systems of donor organ distribution in the world.

DEVELOPMENT PROSPECTS FOR THE SYSTEM OF CADAVER DONOR KIDNEY DISTRIBUTION IN RUSSIA

The Russian national recommendations regarding creation and management of the waiting list for cadaver organ transplants, as well as the algorithm for selecting an optimal donor-recipient pair [7, 33], are aimed at ensuring fair allocation of donor organs to all patients who are in need of such transplants and obtaining the best results after transplantation. According to this document, selection of the donor-recipient pair is carried out taking into account blood group compatibility by the AB0 system, emergency status, anthropometric parameters and the period of being on the waiting list.

Primary selection of the pair is carried out on the basis of the blood group (by the AB0 system) and the result of the cross-match lymphocyte test. At the second stage the patients are ranged by urgent status or need for immediate transplant of several organs (such patients have an absolute priority). At the third stage the choice of a recipient is performed on the basis of histocompatibility with a priority to a minimum number of mismatches in the DR locus. Later patient priority is determined by the 'presence of pre-existing antibodies'. At the same time, despite ET and UNOS principles, recipients who do not have (or have a low level of) pre-existing antibodies have an advantage over patients with pre-existing antibodies (or their high level). At the final stage the candidates are ranged by length of period in the waiting list (candidates who have been waiting for a longer time have a priority). According to the Russian policy cadaver kidneys are allocated only in case of blood group matching.

The main initial stage of donor organ distribution both in the USA (UNOS) and in Europe (ET) for non-sensitized patients is the quality of donor organs. While in Europe the basis is the donor's age, in the USA it is a comprehensive score. We consider the introduction of such a score (or at least a binary feature like in ET) to be an effective measure (probably the most relevant one at the current stage of kidney transplantation development in Russia) which will facilitate an increase in access to transplantation. Patients with diabetes, elderly patients, patients with burdened comorbid medical history should not be limited in access to transplantation. Equity is a basic ethical principle for any donor organ distribution system. On the other hand, it would ensure a most effective organ allocation, enabling to implement the donor kidney potential to a maximum. Patients with the longest predicted life expectancy should receive the best quality kidneys. This approach will ensure an optimal balance between patient-oriented and utilitarian approaches to distribution.

It is evident that in order to implement this approach it is necessary to develop a system of donor organ quality evaluation and a comprehensive assessment of the potential recipient's condition. Recent studies have shown that it can not be achieved by simple copying of the organ distribution system: for example, the effectiveness of the current organ distribution system which has been successfully implemented and is efficiently functioning in the USA may be doubtful in Europe [34].

The most important factors which influence the quality of a donor organ, *relevant for the donor pool in our country,* should be determined. Such factors may be: diabetes mellitus, arterial hypertension, the donor's age, functional condition of the kidneys, the donor's cause of death, type of donor (donor with palpitating heart / asystolic donor). In some cases patient history may be unavailable. Kidneys obtained from such donors may be attributed to a separate category. In this case assessing the quality of the kidneys may be carried out exclusively on the basis of instrumental and laboratory examination data. donor type, cause of death and age.

Assessment of the recipient's condition can present the most difficulties. First of all, prioritization of the recipients may be based either on calculating the predicted life expectancy or on the potential benefit from transplantation (EPTS and LYFT analogs). As a rule, such assessment may be obtained as a result of determining a regression equation which describes a dependency between a certain outcome and a set of predictors with optimal approximation. The set of predictors is determined by the biological significance of the evaluated characteristics and is limited, on the one hand, by the quality of assessing these indicators, and on the other hand – by their relevance for the population under examination.

Absence of contraindications for transplantation is not the only criterion which determines the need for kidney transplantation to a patient with stage 5 CKD (though it is the main one that determines its possibility). The second mandatory condition is confidence that transplantation will lead to increasing the predicted life expectancy or the quality of life. This may be achieved as a result, for example, of studying the connection of quantitative assessment of the comorbid background and the transplantation results in comparison with dialysis treatment and, most importantly, opportunities for using

it to make individual forecasts. Earlier it has been shown by us [9] that deterioration of the comorbid background as a result of prolonged waiting facilitates increased mortality among the recipients after transplantation. This leads to decreased expediency of kidney transplantation as it does not result in a significant improvement in the prognosis versus continuing dialysis treatment.

Calculation of the comorbid background is very important for individual risk assessment (the patient's interests) but also for the development of organ distribution policies (the interests of the supervisory authorities) and for the prioritization of the candidates (the transplantologist's interests). While during inclusion into the waiting list the decision may be made on the basis of a number of binary signs (for example, presence/absence of an infection process, malignant neoplasms or recent myocardial infarction), in order to compare the recipient's comorbid background with the donor organ quality evaluation in an ordinal or interval scale may be required (due to relativity of scales used for comorbid background assessment the possibility to measure the health condition by an absolute scale – a ratio scale – seems quite doubtful even on condition of assuring acceptable equidistance).

Predicted life expectancy calculation (EPTS analogue) seems to us to be a more illustrative and balanced assessment for patients *with a good prognosis*. Even though this assessment is clearly relative, it may have a discreet character (for example, less than 10 years, 10–20 years, over 20 years). At the same time, this may decrease the chances of transplantation for patients in a worse condition. In turn, a ratio showing potential benefit from transplantation (LYFT analogue) during short follow-up time will more likely favour *patients with more severe conditions* [20] for whom the *absolute* life expectancy will increase less compared to a significant *relative* increase of the life expectancy in case of transplantation compared to continuing dialylis treatment. This is due to the fact that patients with a shorter life expectancy achieve their LYFT potential soon after transplantation, while patients with a longer life expectancy achieve their LYFT potential at later stages (10–15 years later).

Patients with diabetes mellitus may be an example. It is known that recipients with diabetes have a significantly shorter life expectancy than patients without diabetes [3–5]. At the same time, younger patients with diabetes mellitus may get a dramatic benefit from kidney transplantation as compared to patients from the same age group without diabetes [18, 20]. This is due to an extremely high mortality among such patients receiving dialysis.

Another example may be patients who need prompt transplantation: their life expectancy after transplantation may be increased by several times as compared to the course of events if they remain on dialysis treatment. However due to the fact that the patients from both examples will have a generally relatively short predicted life expectancy, it would be advisable to perform transplantation of a kidney the functional potential of which will be used up sooner than that of better quality donor kidneys. For a fuller utilization of the donor kidney potential it is necessary to implement an evidence-based system of correlating the surrogate assessment of the patient's condition to the quality of organ which would significantly supplement the uncertain 'old for old' principle.

Thus, the quality of the donor organ should be a key aspect in distribution. For example, when the predicted life expectancy is below 10 years, the kidneys may be allocated taking into account the maximal benefit from transplantation, in case of longer life expectancy – taking into account post-transplantation survival.

Another factor that appears important to us is medical compliance assessment (cognitive disorders, lack of adherence to instructions received from treating physician, missing dialysis procedures, etc.). This factor should also be taken into account in determining candidate prioritization.

Currently we do not have a clear understanding as to how such factors as comorbid background, histocompatibility, waiting period and recipient's age should be introduced into the distribution scheme. We believe that at different times during the waiting period (here it is not the total period of being on the waiting list that should be taken into account but the total length of dialysis treatment) the priority of these factors changes, moreover, in a non-linear manner. Previously [9] we have received strong evidence in favour of this fact: the significance of comorbitity increases along with the increase of the dialysis treatment duration. It is quite possible that in case of long-term waiting the potential benefit from transplantation is significantly decreased even in case of a minimum number of HLA-mismatches. There is also other proof in favour of this assumption [35].

Additional complication is added also by the fact that a significant effect may be due to the interaction of different factors which may be non-linearly related to the outcome probability. For example it is evident that the risk of death for the patient gradually (and probably linearly) increases along with increased age and deterioration of the comorbid background. At the same time the comorbid background will deteriorate (and this means that the risk of death will increase) along with the increase of the waiting time faster in elderly patients as compared to younger ones [36]. Thus even these three factors (age, comorbid background and waiting time) result in a need to include their interaction with corresponding coefficients into the regression model. Adding such an important factor as the presence or absence of diabetes complicates the analysis even further (it is evident that deterioration of the comorbid background in patients with diabetes takes place at a faster rate than in

patients without diabetes). Nevertheless this problem may possibly still be solved. Analysis of the regression equation used to calculate the EPTS rate [37] shows that several predictors are represented by an interaction of different factors. At the same time, a large volume of primary data and the need for mandatory external validation of the model determine the need to solve this problem at the national level. We have been consistently working on this already for several years on the basis of a developed retrospective database. At the same time limited resources and volume of clinical material, as well as its local character indicated a certain bias in the assessment: its result (a draft scheme for donor-recipient pair selection) may be relevant only for our region.

Apart from this, an important aspect of the work which largely determines the opportunities for practical application of its results is the localization of PRA calculations as well as possibility for acceptable mismatches in sensitized recipients. Calculations may be based on the results for a local patient pool or on available data from open sources [38, 39] regarding antigen population frequency in the given region. Such assessments may also have a significant influence on determining priorities among the candidates.

Transplantation probability (not taking into account the comorbidity factor) is a random variable. An important factor which may theoretically influence the priority of the candidates may be the population frequency of antigens which constitute the phenotype and which are taken into account during selection of the pair. It is quite possible that candidates who have a rare HLA phenotype may wait for transplantation for a long time [40–42]. At the same time the waiting time may compensate the impact of this factor in case it is determined that after a certain waiting period the priority of HLA histocompatibility is decreased in favour of other clinical factors (for example the comorbid background).

A disadvantage of the Russian donor kidney distribution system is a lack of a unified waiting list which significantly hinders the choice of an optimal recipient from the point of view of tissue compatibility. Taking into account the territorial peculiarities of our country, the waiting list may be a general one which would unite the efforts of several transplantation centers (not only at the federal region level but by territorial principle). The probability of a center getting a donor organ (for a specific patient) would be determined first and foremost by the size of the local pool of transplantation candidates and would be limited by the possibility to perform a certain number of operations. At the same time the probability of transplantation being performed after receiving a donor kidney is determined by the quality of the waiting list maintenance (updated information about the candidate's condition). Apart from determining the organ distribution policy it is necessary to compare the impact of conservation duration on long-term survival for various quality grafts with the benefit that transplantation with a good immunological background may provide. It may possibly be justified only for candidates whose PRA values are close to 100%.

The fact that under the modern organ distribution system non-sensitized candidates have an advantage over sensitized ones definitely limits the accessibility to transplantation. This may be due to the fact that desensitization of the patients who have pre-existing anti-HLA antigens and are expecting cadaver kidney transplants is not a consistent practice. The results of transplantation in case of pre-existing antibodies may be improved by implementing a virtual cross-match procedure which would take into account the presence of common epitopes [43–45]. Determining acceptable mismatches may significantly improve the results of transplanting kidneys to sensitized candidates [46, 47].

CONCLUSION

Kidney transplantation undoubtedly remains the optimal renal replacement therapy method for the vast majority of the patients. The transplantology and nephrology care environment in our country is gradually changing. This determines the need to adapt and standardize the approaches to the distribution of kidneys obtained from cadaver donors in order to ensure equal access to transplantation for different patients and the maximum fulfillment of their potential. Withdrawing organ allocation from the area of responsibility of the local coordination committees, introducing a unified policy for donor organ distribution and choice of a particular recipient will enable to decrease the bias of the decisions made and possibly improve the transplantation results.

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