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Адрес для корреспонденции:

Россия, 123182, Москва, ул. Щукинская, 1
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Address for correspondence:

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CREATION OF ARTIFICIAL ORGANS AND BIOSYSTEMS: TECHNOLOGIES OF THE FUTURE

Глубокоуважаемые коллеги!

13–14 февраля 2024 года в Москве состоялся второй Форум будущих технологий, обеспечивающий представление передовых научных решений и технологических достижений. Форум будущих технологий проводится в рамках Десятилетия науки и технологий в России, объявленного с 2022 года Указом Президента Российской Федерации Владимира Путина, при поддержке Министерства здравоохранения Российской Федерации, Российской академии наук.

Трансплантация органов сегодня – совокупность передовых высоких технологий сбережения жизни при терминальных стадиях заболеваний. Высокий уровень фундаментальных и прикладных научных исследований в этой области, их направленность на создание конечного продукта – искусственных и биологически синтезированных органов, а также высокотехнологичного оборудования – являются значимым вкладом в укрепление технологического, в том числе биомедицинского, суверенитета Российской Федерации. Перспективы развития трансплантологии в настоящее время предполагают создание портативных и полнофункциональных имплантируемых биологических систем и технических устройств, замещающих функции солидных органов человека. Разработка и внедрение инновационных технологий реабилитации и сохранения донорских органов вне тела человека (ex vivo) создают дополнительные возможности для увеличения числа трансплантаций, улучшения клинических результатов. Отдельное направление – разработка способов достижения активного долголетия исходно обреченных пациентов.

В мире до сих пор не представлена имплантируемая система вспомогательного кровообращения для детей с учетом возрастных и антропометрических особенностей растущего организма. В России в настоящее время завершаются опытно-конст-

Dear colleagues,

On February 13–14, 2024, stakeholders from far and wide gathered in Moscow for the second Future Technologies Forum. The event presented cutting-edge scientific solutions and technological advances. The Forum is held within the framework of the Decade of Science and Technology in Russia (DSTR). The DSTR program was launched in 2022 by the President of the Russian Federation, Vladimir Putin, with support from the Russian Ministry of Health and the Russian Academy of Sciences.

Today, organ transplantation represents a set of advanced high technologies that save lives in end-stage diseases. The high level of fundamental and applied scientific research in this field, their focus on creating the final product – artificial and bioengineered organs, and high-tech equipment – are a major leap towards strengthening Russia's technological and biomedical sovereignty. Prospects for development in transplantology nowadays involve the creation of portable and fully functional implantable biosystems and technical devices that replace human solid organs. Development and implementation of innovative technologies for rehabilitation and preservation of donor organs outside the human body (ex vivo) create additional opportunities for more transplants and better clinical outcomes. A separate direction is the development of ways to achieve active longevity in patients with end-stage conditions.

The world still does not have an implantable ventricular assist device for children, considering the age and anthropometric features of a growing body. In Russia, developmental work is currently being completed and preliminary preclinical trials have begun for an axial pump system (a left ventricular assist device) for patients with small anthropometric parameters, which will help children with incurable cardiovascular diseases.

рукторские работы и начаты предварительные доклинические испытания системы осевого насоса для пациентов с малыми антропометрическими параметрами – искусственного левого желудочка сердца, что позволит помочь детям с инкурабельными заболеваниями сердечно-сосудистой системы.

Перспективы развития трансплантологии предполагают создание систем длительной пресервации, транспортировки и реабилитации донорских органов, разработку уникальных аналогичных систем для педиатрических пациентов, совершенствование методов вспомогательного кровообращения, развитие и внедрение компактных имплантируемых устройств, которые могли бы стать эффективной альтернативой солидным органам человека.

Современная иммуносупрессивная терапия обеспечивает долгосрочную выживаемость пересаженных органов после операции. Альтернативой стандартной иммуносупрессивной терапии являются клеточные биотехнологии, позволяющие замещать иммуносупрессивный эффект протокольной иммуносупрессии без риска развития отторжения и тяжелых побочных эффектов.

Практическую ценность представляют результаты исследования биологических образцов доноров костного мозга, полученные с использованием оригинальной технологии и разработанной платформы, полностью совместимые с международными базами данных о донорах костного мозга. Эволюционирование трансплантационных клеточных технологий в рамках гематологической службы России обеспечит прорыв в развитии клинической медицины в целом.

Форум будущих технологий – ключевое мероприятие для представления передовых научных решений и технологий, которое является чрезвычайно значимым, стратегически необходимым и перспективным событием для отечественной медицины в целом. Достижения в области трансплантологии и искусственных органов занимают достойное место среди прорывных разработок, обеспечивающих развитие и продвижение всей медицинской отрасли на передовой уровень и сбережение населения Российской Федерации.

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

Prospects for the development of transplantology involve the creation of systems for long-term preservation, transportation and rehabilitation of donor organs, development of unique similar systems for pediatric patients, improvement of assisted circulation methods, development and implementation of miniaturized implantable devices that could become an effective alternative to solid human organs.

Modern immunosuppressive therapy ensures long-term organ survival after transplantation. An alternative to standard immunosuppressive therapy is cell biotechnology, which allows replacing the immunosuppressive effect without the risk of developing organ rejection and severe adverse effects.

Of practical value are the results of the study of biological samples of bone marrow donors, obtained using original technology and a developed platform. These results are fully compatible with international bone marrow donor databases. Evolution of transplantation cell technologies within hematology departments in Russia will provide a breakthrough in the development of clinical medicine as a whole.

The Future Technologies Forum is a key event for presentation of advanced scientific solutions and technologies. It is an extremely significant, strategically necessary, and promising event for Russia's healthcare industry as a whole. Achievements in the field of transplantology and artificial organs occupy pride of place among the breakthroughs that are developing and advancing the entire healthcare industry to a state-of-the-art level and saving the lives of the population in the Russian Federation.

Sincerely,
Sergey Gautier,
Fellow, Russian Academy of Sciences
Editor-in-chief, Russian Journal
of Transplantology and Artificial Organs

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LIVER TRANSPLANTATION FOR UNRESECTABLE KLATSKIN TUMOR: FIRST LONG-TERM OUTCOMES – A SINGLE CENTER EXPERIENCE

D.A. Granov, I.I. Tileubergenov, A.R. Sheraliev, V.N. Zhuikov, A.A. Polikarpov, A.V. Moiseenko

Russian Research Center of Radiology and Surgical Technologies, St. Petersburg, Russian Federation

Objective: to demonstrate the first long-term outcomes of treatment of unresectable hilar cholangiocarcinoma (HCCA) after combined neoadjuvant therapy followed by liver transplantation (LT). **Materials and methods.** From 2017 to 2023, at the Russian Research Center of Radiology and Surgical Technologies, 10 patients were included in the treatment protocol for unresectable HCCA. Combined neoadjuvant therapy included endobiliary photodynamic therapy (EPDT), regional chemotherapy (RCT) and systemic polychemotherapy (SPCT). Each modality was applied at least three times over a period of four to six months. Patients were placed on the LT waitlist when tumor marker CA19-9 reduced, there was no radiological evidence of disease progression, and there was no evidence of acute cholangitis. Before LT, the recipients underwent diagnostic laparoscopy to exclude carcinomatosis and also evaluation of regional lymph nodes with urgent morphologic examination. In the absence of extrahepatic tumor spread, LT from a deceased donor was performed according to the classical technique with paracaval and hepatoduodenal lymph node dissection, biliodigestive anastomosis using the Roux-en-Y procedure. The operation was performed in six patients. Patient age ranged from 40 to 55 years (mean, 46.3). The mean time from start of treatment to LT was 9.1 months (range 6 to 14). The mean CA19-9 level at the time of LT was 66.5 IU/mL (8 to 212). **Results.** After combined neoadjuvant treatment, the CA19-9 marker normalized in four patients and there was a 3–4-fold decrease in two patients. Radiological evaluation indicated stable disease in five patients, and a partial response in one. Disease progression was noted in four out of 10 patients. Currently, one of the 6 patients is alive with a follow-up of 34 months. Median (Me) overall survival is 28 months; Me overall survival after LT is 22.2 months; Me survival before progression is 27 months. During long-term follow-up of patients after LT, three patients out of six had disease progression: implantation metastasis (n = 2) at 25 and 27 months follow-up (metastasectomy was performed), carcinomatosis (n = 1) at 20 months follow-up. **Conclusion.** LT for unresectable Klatskin tumor is effective when combined neoadjuvant treatment is used and there is no acute cholangitis. However, the use of endobiliary manipulations (drainage change, EPDT) are risk factors for the development of implantation metastasis.

Keywords: Klatskin tumor, hilar cholangiocarcinoma, liver transplantation, photodynamic therapy, regional chemotherapy.

INTRODUCTION

Hilar cholangiocarcinoma (HCCA), also known as Klatskin tumor, is a malignant tumor arising from bile duct epithelium, localized above the confluence of the cystic duct and up to the level of segmental bile ducts outflow. The disease manifests itself as obstructive jaundice, usually in late stages, which leads to late diagnosis and low survival rate. The best outcomes are demonstrated by definitive surgical intervention in bile duct resection with achievement of negative surgical margin (R0), liver resection with lymph node dissection. However, according to some studies, resectability in hilar cholangiocarcinoma is about 30–50%, 5-year survival rate is not more than 43–67% provided that R0 resection is performed and there are no metastases in regional lymph nodes; 5-year survival in patients with lymph node metastasis is 15–22% [1–5]. Recurrence rate within 5 years reaches 70% [6].

In addition, local relapse occurs in 50% of cases after definitive surgery, and distant tumor metastasis occurs in 30–40% of cases [7]. The use of neoadjuvant therapy (chemotherapy, chemoradiotherapy) before resection may increase achievement of negative surgical margins, but there is no convincing evidence of benefits considering postoperative risks [8–10].

Thus, it should be recognized that resection is currently considered the preferred treatment option when technically feasible. However, it is feasible only for a narrow group of patients, and oncologic outcomes are still unsatisfactory. At the time of treatment, most patients already have unresectable Bismuth–Corlette type 4, 3a, 3b with contralateral lesion of vascular structures (branch of hepatic artery or portal vein) and/or presence

Corresponding author: Aslan Sheraliev. Address: 70, Lenigradskaya str., St. Petersburg, 197758, Russian Federation. Phone: (950) 026-80-74. E-mail: sherali.aslan@gmail.com

of abdominal lymphadenopathy. Such tumor spread does not allow to perform definitive surgery (liver resection in various volumes). For this category of patients, therapy comes down to the use of palliative treatment methods and their combinations: SPCT, RCT, EPDT, brachytherapy/remote-controlled or stereotactic radiotherapy. In the management of such patients, an integral part of treatment is adequate drainage of the biliary tree and monitoring of cholangitis with regular bacteriological examination of bile due to high risk of septic conditions. In some cases, with a proper approach against the background of palliative treatment, it is possible to stabilize the disease by reducing the biological activity of the tumor (decrease in the level of tumor marker CA19-9).

In this situation, LT can be considered as a definitive treatment option for patients with inoperable HCCA due to complete removal of tumor tissue and the whole organ with potential macroscopically non-visualized micrometastasis and a substrate for relapse. However, based on analysis of available studies, the best outcomes of LT for Klatskin tumor can only be achieved with strict patient selection in combination with neoadjuvant treatment [11]. For example, more recent publications of the Meyo Clinic treatment protocol demonstrate a 5-year survival rate of 72% [12]. Thus, a combination of neoadjuvant therapy with subsequent LT for unresectable Klatskin tumors is a very promising therapeutic option.

MATERIALS AND METHODS

From 2017 to 2023, 10 patients were included in the developed protocol (Fig. 1) for the treatment of unresectable HCCA followed by LT at Russian Research Center of Radiology and Surgical Technologies (Table).

The unresectability criterion was lesion of segmental bile ducts – Bismuth–Corlette type 4, 3A, 3B with contralateral lesion of vascular structures (branch of hepatic artery or portal vein). The clinical stage of the disease was established via computed tomography (CT), magnetic resonance imaging (MRI), and direct cholangiography.

Patients with a tumor size <5 cm and localization above the cystic duct were considered. Distant metastases were excluded by radiologic examination methods. In all cases, histological confirmation by intraductal punch biopsy, assessment of CA19-9 level (in the absence of active cholangitis) before treatment, regular bacteriologic examination of bile and appropriate antibacterial therapy were mandatory. A combination of EPDT, RCT and SPCT was used as neoadjuvant therapy (Fig. 1).

Each technique was used at least three times over a period of three to eight months with radiological evaluation and CA19-9 level determination to monitor tumor growth and biological activity. Patients were placed on the LT waiting list only when the tumor marker decreased, no radiologic evidence of disease progression, and

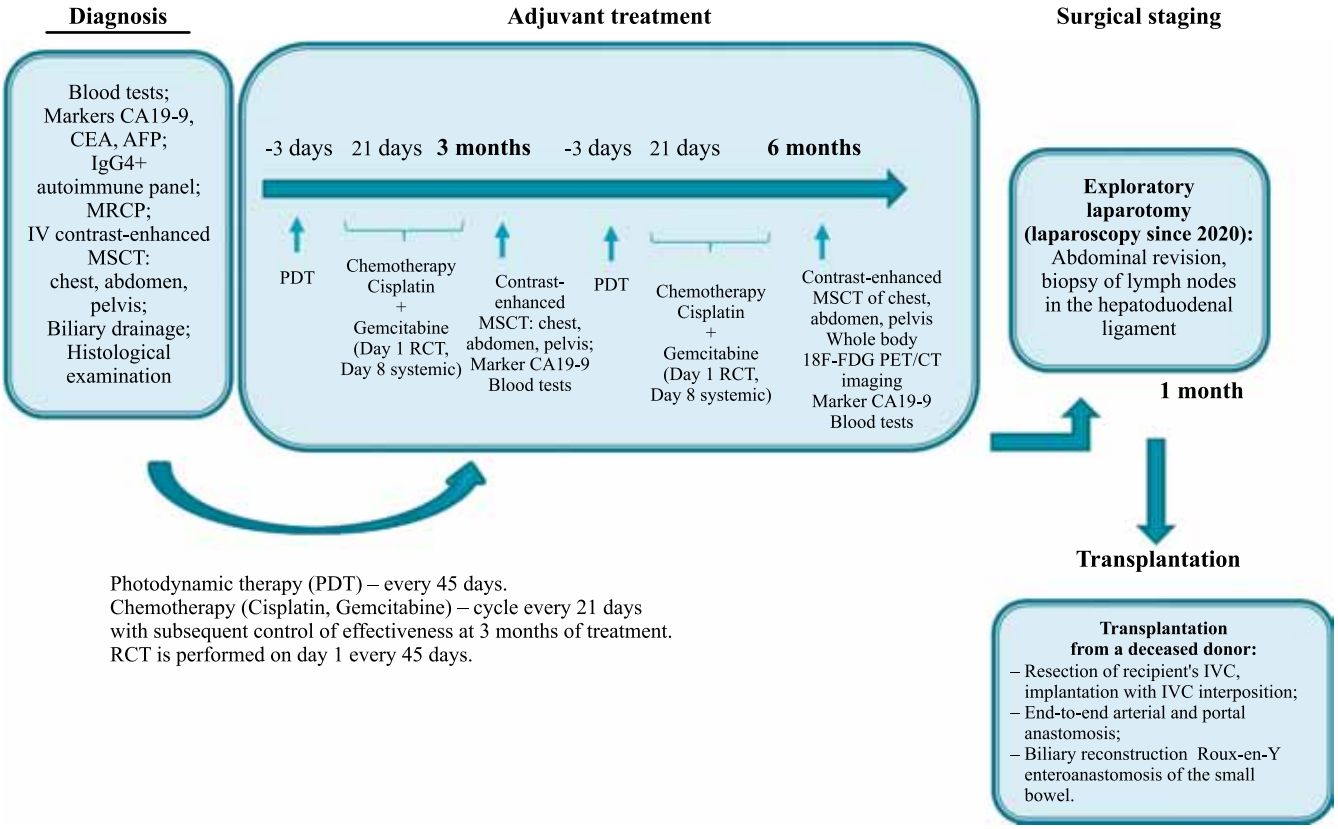

Fig. 1. Brief description of the multidisciplinary protocol for treatment of unresectable Klatskin tumor with subsequent liver transplantation, developed at the Russian Research Center of Radiology and Surgical Technologies

no acute cholangitis. Whole body 18F-FDG positron emission tomography (PET) imaging was performed to monitor tumor metabolic activity and to rule out extrahepatic spread. Before LT, the potential recipient underwent laparoscopic abdominal exploration for carcinomatosis and evaluation of hepatoduodenal lymph nodes with excision of suspicious tissue for morphologic examination. LT was not performed where extrahepatic spread was histologically confirmed. Otherwise, LT was performed according to the classical technique with paracaval and hepatoduodenal lymph node dissection, biliodigestive anastomosis using the Roux-en-Y procedure. All suspicious (enlarged/dense) lymph nodes around the hepatoduodenal ligament, celiac artery, aorta and inferior vena cava, were removed. According to the Japanese Research Society for Gastric Cancer (JRSGC) classification, this corresponds to anatomic groups 5, 7, 8a, 8p, 9, 12a, 12b, and 12p.

The combined neoadjuvant treatment protocol initially included 10 patients. LT was performed in 6 patients, including 2 females and 4 males. The age of the patients ranged from 40 to 55 years (mean, 46.3). The mean time from treatment initiation to transplantation was 9.1 months (range, 6 to 14). The mean CA19-9 level at the time of transplantation was 66.5 IU/mL (range, 8 to 212). A standard triple-drug immunosuppression protocol (tacrolimus, mycophenolic acid, prednisolone) was used in the early postoperative period, followed by conversion from tacrolimus to an mTOR inhibitor (everolimus) after one month.

RESULTS

Despite neoadjuvant therapy, three patients showed a more than twofold increase in CA19-9 levels over an average of four months. In two of them, CT revealed disease progression according to the RECIST criterion. In the remaining two patients, carcinomatosis was detected during diagnostic laparoscopy before the planned LT, which was the reason for exclusion from the LT waitlist.

Using a combination of methods (EPDT, RCT, SPCT) as neoadjuvant treatment, we managed to normalize CA19-9 levels in four patients and achieve 3–4-fold decrease of this tumor marker level in two patients. Diagnostic laparoscopy and biopsy of hepatoduodenal lymph nodes in all patients with decreased CA19-9 levels revealed no metastases, which allowed for LT (Table).

During the follow-up period, we analyzed such indicators as overall survival (OS), from the time of neoadjuvant treatment initiation to the time of death in the post-transplant period; OS after LT; and progression-free survival after liver transplantation (PFS after LT).

When analyzing the OS from the moment of neoadjuvant treatment and after LT, one patient out of 6 patients is alive presently, with a follow-up period of 34 months. The median OS was 28 months (Fig. 2).

Median OS after LT was 22.2 months (Fig. 3).

After LT, the disease progressed in three patients out of six (Fig. 4). Two patients had implantation metastasis at 25 and 27 months of follow-up. One patient had carcinomatosis at 20 months follow-up. Median PFS was 27 months.

Two patients with signs of implantation metastasis (right hypochondrium, anterior abdominal wall) were subsequently operated upon (metastasectomy). Detailed description of the clinical cases is presented below.

Case #1

Patient D., 55 years old, in November 2020, the disease manifested with obstructive jaundice; external-internal bile duct drainage was performed. The jaundice

Table

Treatment outcomes with tumor marker dynamics, RECIST and survival rates for all patients included in the developed protocol

Patient	Age (years)	No. of EPDTs	No. of RCTs	No. of SPCT	Ca19-9 before treatment	Ca19-9 after treatment/ at the time of LT	RECIST	Time to progression/LT	Survival after LT	Survival from start of treatment
1	49	7	11	8	986	8	CR	**LT after 14 months**	36 months	50 months
2	40	4	4	5	754	24	SD	**LT after 8 months**	35 months	43 months
3	37	4	4	4	337	754	SD	Carcinomatosis at diagnostic laparoscopy	–	11 months
4	56	2	2	3	3416	7256	PD	Progression after 4 months	–	7 months
5	55	4	3	5	864	212	SD	**LT after 6 months**	28 months	34 months
6	46	5	6	6	789	1456	PD	Progression after 5 months	–	8 months
7	42	3	3	4	62	3.3	SD	**LT after 8 months**	1 months	9 months
8	37	3	3	4	515	150	SD	**LT after 7 months**	22 months	28 months
9	55	2	2	1	420	2	SD	**LT after 12 months**	4 months	16 months
10	34	1	1	1	474	19	SD	Carcinomatosis at diagnostic laparoscopy	–	7 months

was stopped. Based on examination results, unresectable HCCA was diagnosed. Tumor marker CA19-9 before treatment was 864 IU/ml. Cholangiography and intraductal punch biopsy were performed. Cholangiography showed that the tumor involved the right and left hepatic ducts, which corresponded to Bismuth–Corlette type 4. The tumor was histologically verified: moderately differentiated bile duct adenocarcinoma. Given the absence of signs of distant metastasis and lymph node involvement, the patient was considered as a potential LT candidate and was included in the waiting list. Neoadjuvant treatment was performed according to the treatment protocol we developed. The patient received 4 EPDT procedures, 3 RCT cycles and 5 SPCT cycles. After preliminary intraoperative staging, in 6 months from the moment of inclusion in the waiting list, the patient underwent LT from a deceased donor in May 2021. At the time of surgery, tumor marker CA19-9 decreased 4-fold (212 IU/ml). One month after transplantation, there was a clinical and laboratory picture of graft rejection. An ultrasound-guided liver trephine biopsy was performed. Histological conclusion: cholestasis, graft rejection. Blood biochemistry tests dated June 29, 2021 showed increased levels of total serum bilirubin (181.5 μmol/l), alanine aminotransferase 1630 units/L, aspartate aminotransferase 736 units/L, lactate dehydrogenase 426 units/L, and alkaline phos-

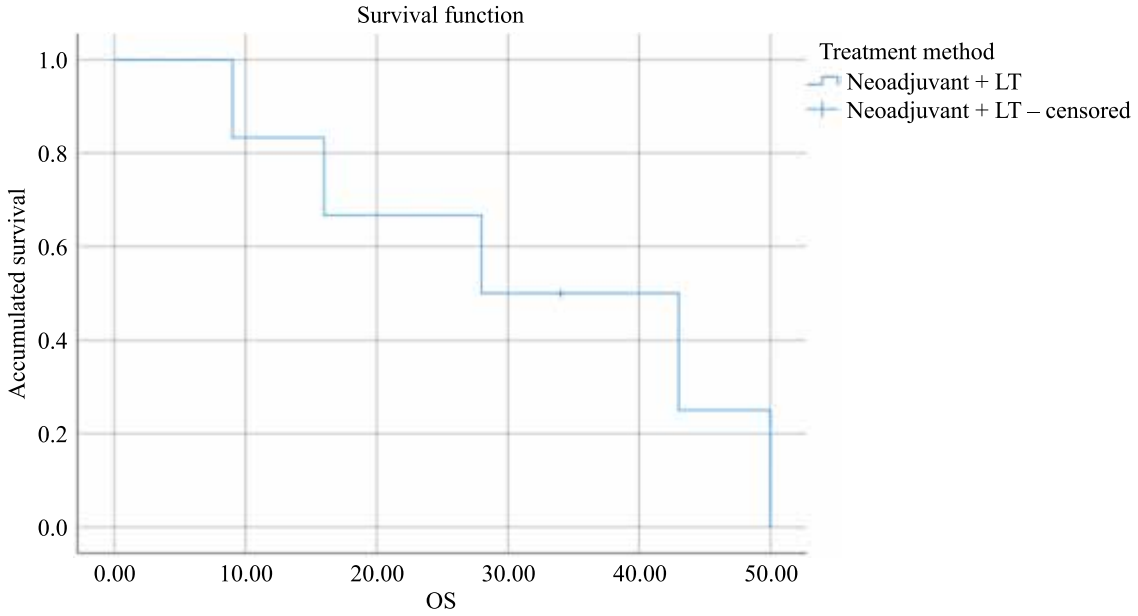

Fig. 2. Overall survival from start of neoadjuvant treatment to time of death in the post-transplant period

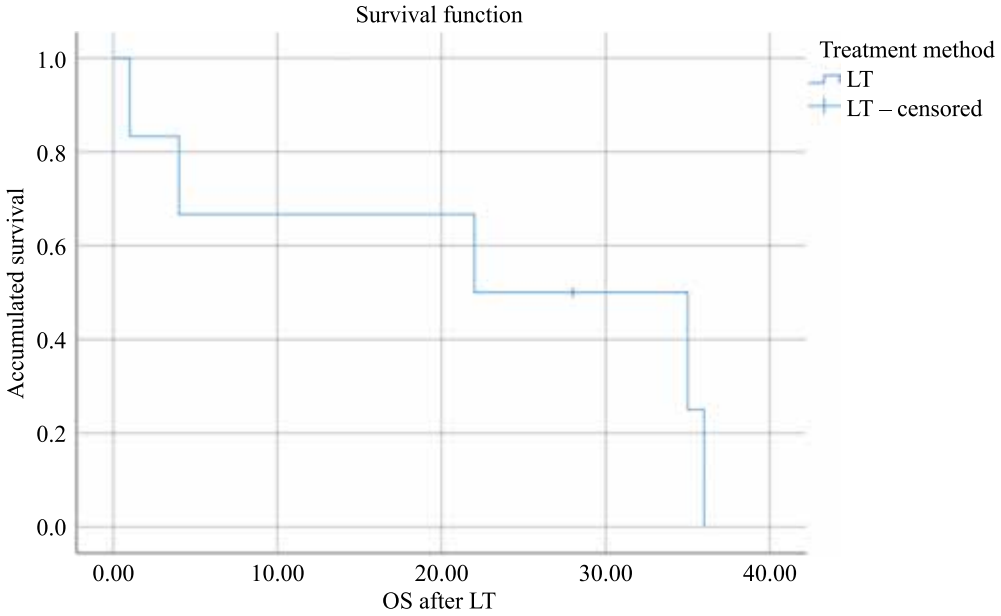

Fig. 3. Overall survival (OS) after transplantation

phatase 225 units/L. Immunosuppressive therapy was corrected (everolimus-to-tacrolimus conversion), pulse methylprednisolone therapy without significant correction of biochemical parameters of the graft. Five sessions of high-volume plasma exchange were performed with positive dynamics – relieving graft rejection signs. Considering the patient's underlying disease, immunosuppression was supplemented with an mTOR inhibitor (everolimus). After 25 months of follow-up after LT, at the next visit to the hospital, the patient was found to have elevated levels of tumor marker CA19-9 (from 38 to 199 IU/mL). Whole body 18F-FDG PET/CT imaging was performed on July 7, 2023. Findings: metabolic active thickening in the 7th intercostal space on the right side, a focus of radiopharmaceutical hyperfixation in the liver at the level of this tumor SUV 4.7, size 22 × 19 × 21 mm (Fig. 5). The finding was considered as an implantation metastasis of HCCA in the area of percutaneous cholangiodrainage previously performed before LT.

The patient underwent surgical intervention (August 30, 2023), which included excision of metastatic lesion of intercostal muscles of the anterior chest wall on the right side, diaphragm, marginal atypical liver resection, drainage of the right pleural cavity using the Bülau

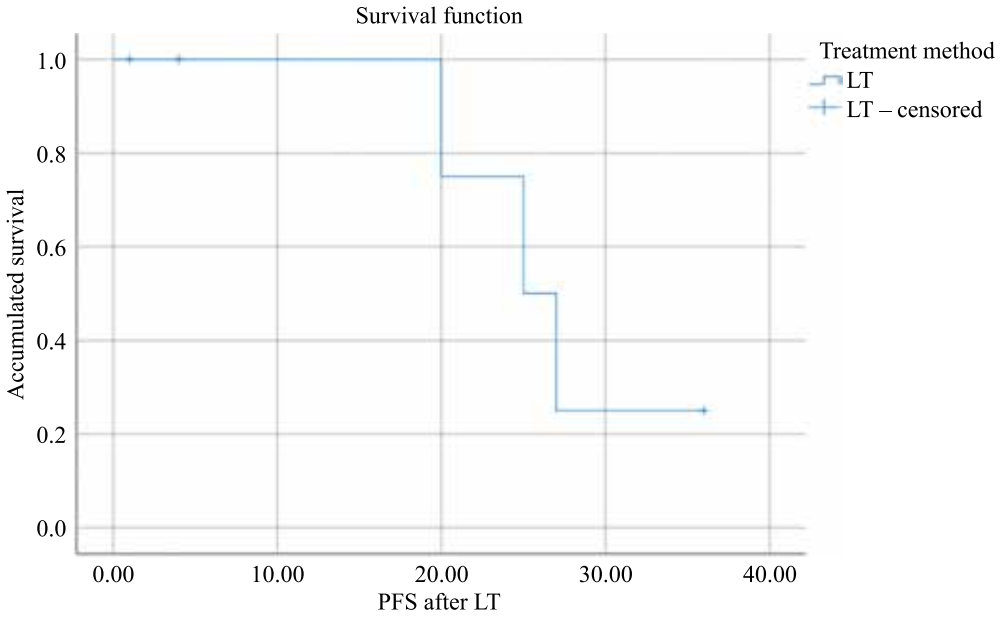

Fig 4. Progression-free survival (PFS)

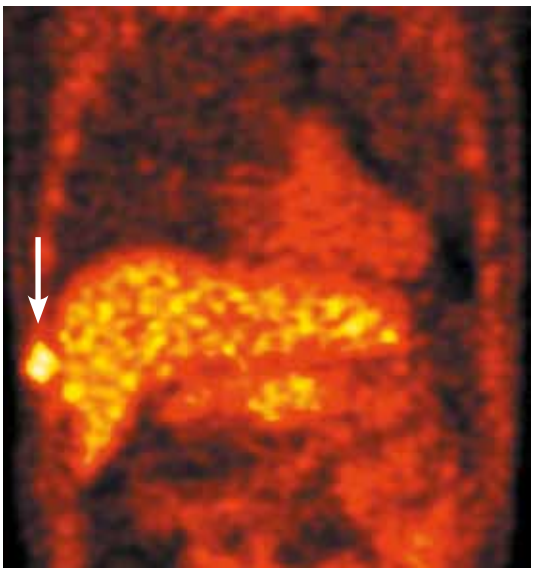

Fig. 5. Whole body 18F-FDG PET/CT imaging at 25 months of LT (study dated July 7, 2023). The CT scan shows a metastatic focus with active accumulation of radiopharmaceuticals (marked with a white arrow)

drain procedure (Fig. 6). The postoperative period was uneventful. Histological study of postoperative material: metastasis of poorly differentiated adenocarcinoma into subcutaneous fatty tissue and transverse striated muscle tissue. Liver fragments were with pronounced artificial changes without convincing signs of tumor growth. Considering the definitive nature of the surgical intervention, absence of tumor spread to the liver, it was decided not to perform systemic chemotherapy and radiotherapy. The patient continues to be monitored. Indicators of tumor markers as of November 13, 2023 are within normal values (CA19-9 23.7 IU/mL, carcinoembryonic antigen 7.3 ng/mL).

Case #2

Patient Y., 40 years old, the disease manifested with obstructive jaundice, fever, acute cholangitis in February 2017. Percutaneous external-internal drainage of bile ducts was performed. After additional examination, CT

magnetic resonance imaging revealed a 30 × 40 mm tumor in the porta hepatis.

In March 2018, after stopping the infectious-inflammatory process and obstructive jaundice, surgical treatment was undertaken, exploratory laparotomy was performed during which the tumor process was considered as unresectable – invasion of the native hepatic artery, Bismuth–Corlette type 4. A biopsy was performed. Histological conclusion – highly differentiated pancreaticobiliary adenocarcinoma. Given the unresectable nature of the tumor, absence of distant metastasis and regional lymph node involvement, the patient was included in the LT waitlist. The CA19-9 level at the moment of treatment initiation was 754 IU/ml. The patient received neoadjuvant treatment according to the protocol we developed. The patient received 4 EPDT procedures, 4 RCT cycles and 5 SPCT cycles. After 8 months from the moment of inclusion in the waiting list, the patient underwent LT from a deceased donor in February 2020. At the time of surgery, tumor marker CA19-9 was normalized (24 IU/ml). The postoperative period was uneventful, the patient was regularly observed in the hospital. In 27 months after LT, during the next visit, a CT scan revealed a tumor on the anterior abdominal wall on the right side in the projection of the postoperative scar, measuring up to 51 × 56 × 75 mm, with invasion of the anterior abdominal wall muscles over the entire thickness, the tenth rib, the S6 liver capsule throughout 10 mm, involving the ascending colon (Fig. 7).

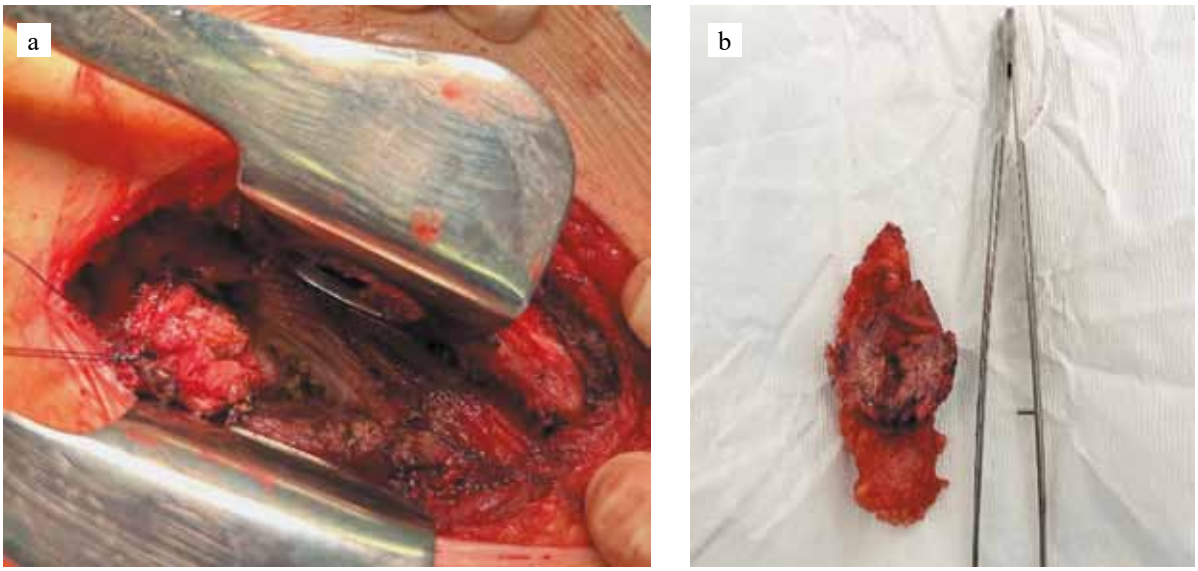

Fig. 6. Intraoperative photograph: a, the tumor node is taken on a holder; b, macroscopic specimen of the removed metastasis

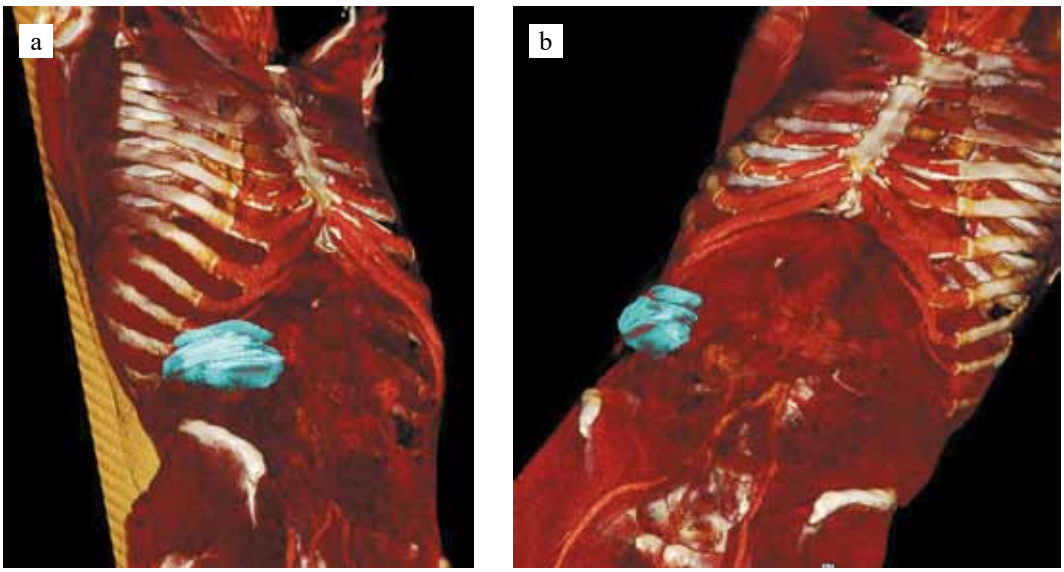

Fig. 7. Preoperative 3D reconstruction of CT tumor images with assessment of invasion of deep tissues and structures (turquoise)

The operation was performed on May 27, 2022, and included: removal of tumor from the anterior abdominal wall, resection of the tenth and eleventh ribs on the right side, marginal atypical liver resection, right hemicolectomy, reconstruction of the anterior abdominal wall with plasty using a Permacol biological implant (Fig. 8). The removed tumor conglomerate is shown in Fig. 9.

Histological examination of the removed tumor: moderately differentiated adenocarcinoma without signs of dMMR/MSI-H. Testing positive to PMS2, MLH1, MSH2, and MSH6 in the tumor. In the postoperative period after wound healing, the patient received 3 SPCT cycles based on the GemCis regimen. Four months after metastasectomy, the patient died of a stroke and autopsy showed no signs of progression. Overall survival after LT was 35 months, and from the time of neoadjuvant treatment 43 months.

DISCUSSION

LT as a therapeutic option for HCCA patients was attempted earlier (1980–1990); despite the reasonable potential advantage of definitive removal of the affected organ with achievement of a negative resection margin, its outcome left much to be desired. In the early days of attempts to address this problem, hospitals performing LT for HCCA reported a 3-year survival rate of about

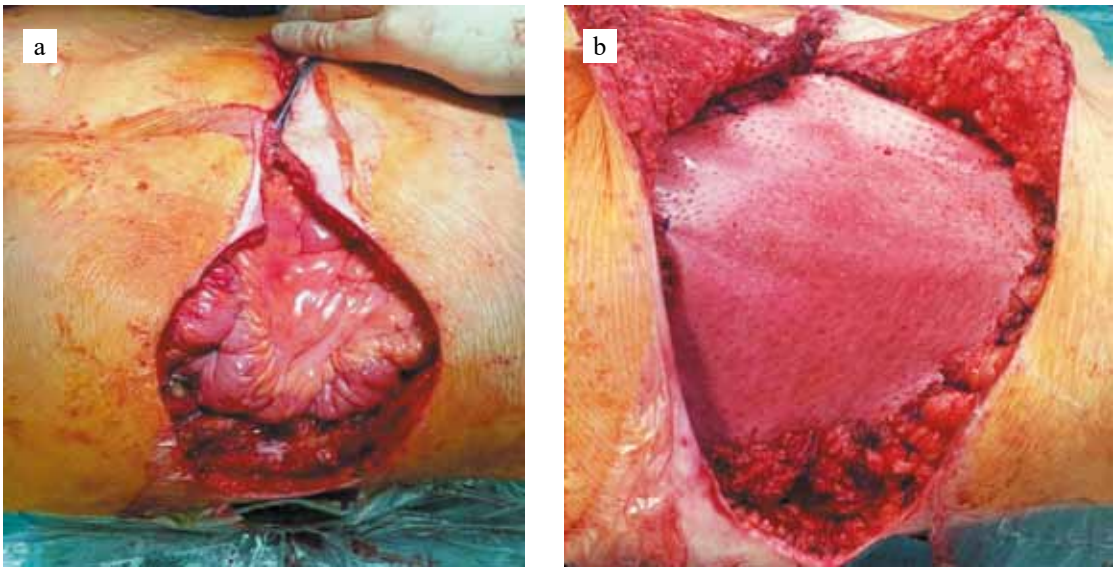

Fig. 8. Photograph after tumor removal: a, there is a defect in the anterior abdominal wall; b, photograph after repair of the anterior abdominal wall defect with biological implant Permacol. The synthetic material is sewn using single interrupted sutures to the edges of the muscles and anterior abdominal aponeurosis

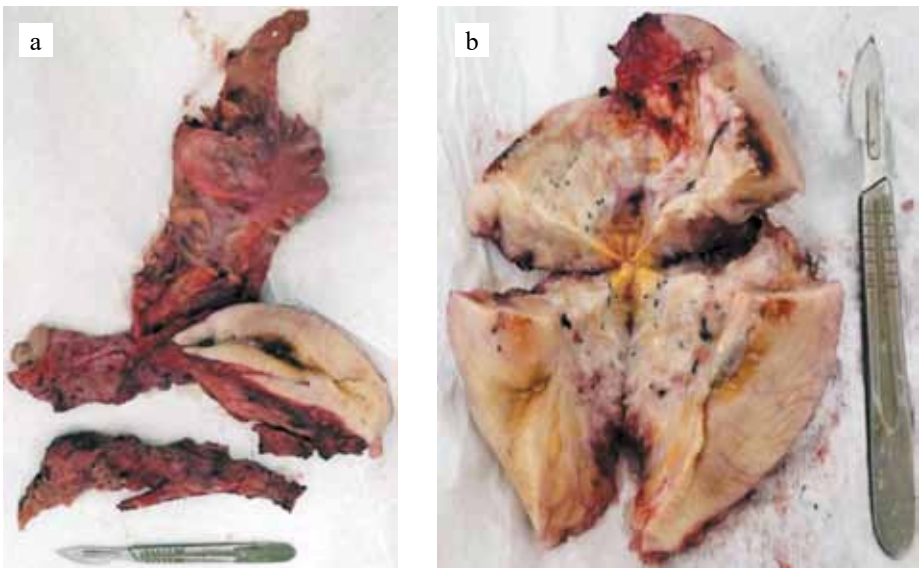

Fig. 9. Photograph of removed tumor: a, general view of the removed tumor with resected structures: anterior abdominal wall, rib, colon segment; b, view of the anterior abdominal tumor after incision

30% [11]. These results led to the conclusion that LT alone does not improve long-term treatment outcomes. Moreover, immunosuppression is known to increase the risk of tumor progression and may lead to rapid patient death. However, careful analysis of accumulated data has revealed that the cohort of patients with negative resection margins and no metastases in regional lymph nodes had much better survival rates. In addition, a small group of patients at the Mayo Clinic who received only chemoradiotherapy without subsequent surgical treatment had a 22% 5-year survival rate [13]. Unsatisfactory outcomes of standard methods of HCCA treatment and the success of individual studies were the reason for the active application of combined methods of treatment. Given evidence on the efficacy of chemoradiotherapy for HCCA and the predominant progression of the disease in the form of local recurrence rather than distant metastasis, a group of Nebraska transplant surgeons first developed a strategy for neoadjuvant high-dose-rate (HDR) brachytherapy in combination with chemotherapy with 5-fluorouracil (5-FU) and subsequent LT [12, 14]. The use of HDR brachytherapy increased the incidence of biliary, infectious and vascular complications. Still, early results were promising with respect to reducing local recurrence. The Mayo Clinic subsequently adopted this concept, developing a similar protocol for neoadjuvant therapy followed by LT in 1993. The protocol combined the benefits of radiotherapy, chemotherapy, and LT while carefully selecting patients with localized, unresectable HCCA. Preliminary results for 11 patients, which were reported in 2000, were encouraging, and an update in 2004 reported an 82% 5-year survival rate in 28 patients [7]. However, the survival rate fell to 72% as the patient sample increased [12].

Unfortunately, domestic experience with LT in HCCA is very limited, judging by the lack of significant publications. Treatment of technically unresectable HCCA is classified as palliative, and its outcomes and prognosis do not differ much from those of disseminated process and, as a rule, are caused by rapidly progressing biliary obstruction and cholangitis. The primary task in the management of such patients is biliary decompression to stop obstructive jaundice and purulent cholangitis signs [15]. Transhepatic percutaneous cholangiostomy is the method of choice for biliary decompression for this patient category due to the impracticability of retrograde drainage in more than a half of cases with proximal extrahepatic bile duct strictures [16].

The standard of antitumor treatment for unresectable HCCA, as well as for any form of inoperable locally advanced or metastatic cholangiocarcinoma according to Russian and foreign clinical guidelines is SPCT based on GemCis (gemcitabine plus cisplatin) or GemCap (gemcitabine plus capecitabine) regimens, as well as stereotactic precision conformal chemoradiotherapy with fluoropyrimidines [17, 18] or other chemotherapy and radiotherapy variants depending on the patient's somatic status, individual intolerance and developing complications.

Moreover, according to combined statistics on the effectiveness of these methods of treatment for all inoperable malignant biliary tumors, the median overall survival rate is 8–10 months [19]. Some of the best outcomes achieved using chemoradiotherapy demonstrate a 4-year survival rate of 30% [20].

EPDT is a relatively new progressive treatment option for unresectable HCCA . The efficacy of EPDT in combination with biliary decompression is confirmed by numerous studies, some of which showed a five-fold difference in life expectancy [15, 21–25].

Having a wide enough experience in hepatobiliary surgery and oncology in general, as well as in HCCA therapy in particular, we tried to use the whole available arsenal of possibilities in relation to this pathology. Like most colleagues, we actively perform percutaneous transhepatic cholangial drainage for biliary decompression, with compulsory assessment of the bacteriologic flora of bile and antibacterial therapy according to sensitivity. The presence of percutaneous transhepatic drains in the biliary tree in HCCA patients implies the relative ease of EPDT delivery to the lesion area and the possibility of repeating the procedure many times, which confirms our own experience [15, 16].

The ideological similarity between the world-renowned protocol of the Mayo Clinic and the treatment protocol we have developed is stopping tumor growth, reducing the biological activity of the tumor until the time of definitive treatment. Our neoadjuvant treatment includes EPDT and no radiotherapy. Undoubtedly, the effectiveness of LT in unresectable Klatskin tumor is beyond doubt, however, as the authors themselves admit, remote-controlled radiotherapy and intraductal brachytherapy is often accompanied by severe cholangitis, biliary abscesses, sepsis and vascular complications [7, 12], which, in our opinion, is due to the pronounced connective tissue overgrowth and formation of rough scar structures in the hepatoduodenal ligament. This cannot but affect the intraoperative precision of dissection of anatomical structures and formation of anastomoses, which can significantly complicate the vascular reconstruction procedure during LT. Thus, after our combined neoadjuvant treatment, there were no postoperative vascular and biliary complications. However, it is necessary to recognize that multiple endobiliary interventions (the need to change drains, multiple EPDT procedures) are risk factors for implantation metastasis. Thus, in our study, two patients developed implantation metastases in the projections of previously installed biliary drains during the long-term follow-up period. The necessity to maintain a balance between the benefit and possible complications leaves open the question of using radiotherapy/brachytherapy. Most likely, the use of simultaneous bile duct drainage and brachytherapy at the first stages of

neoadjuvant treatment can reduce the likelihood of implantation metastasis [26]. With regard to chemotherapy, in our opinion, in addition to using SPCT, transcatheter arterial chemotherapy infusion (TACI) allows to create a high concentration of chemotherapy drug in a limited anatomical area, thereby increasing cytostatic effect and reducing overall toxicity. In addition, direct angiographic examination allows one to visually assess the degree of involvement of vascular structures in the tumor process. Alternating SPCT and TACI with EPDT sessions, in our opinion, seems to be the most optimal option for neoadjuvant therapy, considering the resulting decrease in tumor biological activity, absence of an increase in the risks of biliary and vascular complications in the post-transplant period.

An additional advantage of a neoadjuvant protocol is the "test of time", as a cohort of patients with aggressive tumor biology experience disease progression despite treatment [27]. In such cases, LT is not indicated.

CONCLUSION

Based on our own and foreign experience, we conclude that indications for LT and its success in unresectable Klatskin tumor depend on careful selection of patients according to strict inclusion and exclusion criteria, on effectiveness of combined treatment methods for at least 3–4 months through reduction of the biological and metabolic activity of the tumor, reduction of the size, as well as assessment of metastatic involvement of lymph nodes, assessment of extrahepatic spread and monitoring of acute cholangitis.

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The authors declare no conflict of interest.

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EFFECT OF DELAYED GRAFT FUNCTION ON IMMEDIATE AND LONG-TERM KIDNEY TRANSPLANT OUTCOMES

A.V. Shabunin[1, 2], P.A. Drozdov[1], I.V. Nesterenko[1], D.A. Makeev[1], S.A. Astapovich[1], O.S. Zhuravel[1, 2], L.R. Karapetyan[1]

[1] Botkin Hospital, Moscow, Russian Federation
[2] Russian Medical Academy of Continuous Postgraduate Education, Moscow, Russian Federation

Objective: to analyze the immediate and long-term outcomes of kidney transplantation (KT) depending on the duration of delayed graft function (DGF). **Materials and methods.** The study conducted a retrospective analysis of KT outcomes in 312 patients operated on at Botkin Hospital from June 2018 to December 2022. Exclusion criteria were primary non-function, severe surgical complications that required emergency transplantectomy in the first week after KT and cases where a comprehensive approach to DGF prevention was applied. DGF was defined as the need for dialysis within the first 7 days of KT. The severity of this complication was assessed by the time it took the transplanted kidney function to normalize from mild DGF to severe. We analyzed the immediate and long-term outcomes of KT depending on the presence of initial function and the severity of DGF. **Results.** DGF developed in 25.3% of cases. The mean time for graft function normalization was 16.5 ± 6.8 days. Mild DGF occurred in 68% of cases, severe DGF was determined in the remaining cases (32%). The incidence of complications was statistically significantly higher in the severe DGF group: 14/25 (56%) *vs.* 15/54 (27.8%) ($p = 0.047$). There were also no significant differences in the rate of complications between recipients with immediate and mild DGF: 43/233 (18.4%) *vs.* 15/54 (27.8%) ($p > 0.05$). Severe DGF lasting for more than 2 weeks had a statistically significant association with postoperative complications ($p = 0.047$) and with decreased long-term graft survival (log-rank $p = 0.021$). **Conclusion.** Development of severe DGF mainly depends on donor characteristics, timing and peculiarities of graft preservation. Nevertheless, other factors, such as acute calcineurin inhibitor nephrotoxicity, should not be ignored. Therefore, prevention of all potentially modifiable risk factors for DGF should go hand in hand with the expansion of the indications for donation.

Keywords: kidney transplantation, delayed renal graft function, survival.

INTRODUCTION

KT is currently the most optimal modality of renal replacement therapy (RRT), as it is associated with the best long-term survival outcome and improved quality of life [1]. In this regard, the KT waiting list is steadily expanding in the world and in the Russian Federation, despite the annual increase in the number of transplants performed [2, 3]. This fact dictates the need for a constant expansion of the indications for deceased organ donation to increase the number of renal transplants. At the same time, this approach increases the risk of DGF, which leads to poorer immediate and long-term outcomes of KT [4].

DGF is defined as the need for RRT within 7 days after transplantation [5–6]. Many authors have noted a connection between DGF and several long-term adverse effects, including acute rejection, decreased graft survival and others [7–13], making the relevance of this problem extremely high. Our previous study demonstrated a statistically significant decrease in long-term survival of grafts that underwent delayed function. At the same time, DGF is certainly a multifactorial problem, and not all conditions falling under the classical definition of this complication may have an impact on early and long-term outcomes of KT. Thus, this paper is devoted to analyzing the immediate and long-term outcomes of KT depending on the duration of DGF.

MATERIALS AND METHODS

The study was based on a retrospective analysis of KT outcomes in 312 patients operated at Botkin Hospital from June 2018 to December 2022. Patients with primary non-function were excluded, as well as those with severe surgical complications that required urgent graftectomy in the first week after KT. Since mid-2022, a set of measures aimed at preventing DGF has been proposed and implemented in our hospital. It allowed us to significantly reduce the burden and slightly change the structure of risk factors for this complication. In order to exclude errors in interpretation of the results of this analysis, cases where a comprehensive approach to DGF prevention was applied, were also excluded.

Mean recipient age was 46.02 ± 11.5 (22 to 67) years. There were 196 (62.8%) men, 116 (37.2%) women. Most

Corresponding author: Pavel Drozdov. Address: 15/8, Brusilov str., Moscow, 117148, Russian Federation.
Phone: (962) 985-04-41. E-mail: dc.drozdov@gmail.com

of the patients were on RRT: 212/312 (67.9%) were on hemodialysis, 38/312 (12.2%) were on peritoneal dialysis; and 15 patients (4.8%) were operated on before RRT was initiated. Among the causes of end-stage kidney disease, chronic glomerulonephritis was predominant – in 173/312 (55.4%). Also, 26/312 (8.3%) had diabetic nephropathy, 25/312 (8%) had chronic tubulointerstitial nephritis, 15/312 (4.8%) had chronic pyelonephritis, and 9/312 (2.8%) had a renoprival condition and 64/312 had other diseases (20.5%).

Isolated KT from a deceased donor was performed in all cases. Mean donor age was 48.35 ± 10.2 (18 to 71) years. In 163 (52.2%) cases, the donor was considered a standard donor, 137 (43.9%) cases used expanded criteria donors, and 12 (3.8%) cases used grafts obtained from donors with irreversible circulatory arrest. Mean donor ICU stay was 61.7 ± 37.2 (95% CI: 36.9–86.5) hours and mean cold preservation time was 11.3 ± 4.9 (95% CI: 10.1–13.3) hours.

Kidney removal, kidney transplantation, management of recipients in the early postoperative period, and selection of immunosuppressive therapy were all done in accordance with standard protocols of the National Clinical Guidelines. DGF was defined as the need for hemodialysis within 7 days after surgery. The severity of this complication was assessed by the time it took to normalize graft function. In this work, we used the following gradation: mild DGF (≤14 days) and severe DGF (≥15 days). We analyzed the immediate and long-term outcomes of KT depending on initial graft function and DGF severity.

Statistical processing and data analysis

Statistical processing and data analysis were performed in the SPSS Statistics program for Microsoft Windows version 26 (USA). Student's t-test or Welch's t-test was used to compare two groups of quantitative data in the normal distribution (depending on the equality of variances). When distribution differs from normal, the Mann–Whitney U-test was used to compare two groups of quantitative data, and the Kruskal–Wallis test was used to compare three or more groups. Qualitative indicators were compared using Pearson's chi-squared test or Fisher's exact test with determination of the odds ratio (OR) and closeness of association of the studied features. Survival analysis was performed using the Kaplan–Meier estimator with determination of statistically significant differences using the Cox–Mantel log-rank test. Differences were considered statistically significant at $p < 0.05$, the trend toward statistical significance was defined as $p < 0.1$.

RESULTS

Of the 312 cases retrospectively selected for this analysis, DGF occurred in 79 (25.3%). The mean time to normalization of graft function was 16.5 ± 6.8 (95% CI: 10.2–21.7) days. According to the above grading of DGF severity, 54/79 cases (68%) were determined to have mild DGF, and the remaining cases had severe DGF (25/79, 32%).

In 72/312 (23.1%) cases, surgical complications of varying severity developed in the early postoperative period. Postoperative wound hematoma, requiring revision, developed in 18/312 (5.7%) cases, lymphocele requiring intervention in 25/312 (8%), wound infection in 27/312 (8.6%), urological complications in 11/312 (3.5%), pneumonia in 6/312 (1.9%), and sepsis in 15/312 (4.8%) recipients. In 23/312 (7.3%) patients, two or more complications simultaneously (or sequentially) were recorded in the early postoperative period. In 7 cases, these complications led to graft loss, in 3 of which the cause was in-hospital recipient mortality. DGF increased the chances of surgical complications by 2.56 (95% CI: 1.5–4.5) times ($p = 0.001$). The rate of complications was statistically significantly higher in the severe DGF group: 14/25 (56%) versus 15/54 (27.8%) in mild DGF ($p = 0.047$). It should be noted that we did not find statistically significant differences in the incidence of complications between recipients with immediate and mild delayed function: 43/233 (18.4%) vs. 15/54 (27.8%) ($p > 0.05$). Immediate KT outcomes depending on initial graft function are clearly presented in Table.

One-year kidney graft survival in a group of 312 recipients was 92.4% (95% CI: 88.1–96.3%) and four-year survival was 74.0% (95% CI: 63.2–81.2%). DGF worsened the long-term outcomes of KT statistically significantly, with cases of death of a recipient with a functioning graft censored. Thus, the 1- and 4-year survival rates were 99.4% (95% CI: 91.3–100%) and 95.5% (95% CI: 82.3–98.1%) for immediate graft function, and 94.8% (95% CI: 87.4–97.2%) and 83.6% (95% CI: 71.1–92.4%) for DGF (log-rank $p = 0.001$). However, long-term survival between immediate function and mild DGF recipients was not statistically significantly different ($p > 0.05$). In contrast, long-term graft survival for severe DGF was statistically significantly lower (log-rank $p = 0.021$) than immediate function. The 1- and 3-year graft survival rates for severe DGF were 79.4% (95% CI: 69.2–85.4%) and 53.0% (95% CI: 26.5–71.2%), respectively. The main causes of graft loss in the severe DGF group, in addition to recipient death for reasons unrelated to the transplanted kidney, were infectious complications 16/25 (64%) and acute rejection 9/25 (36%). An analysis of graft survival depending on initial function is presented in Figure.

DISCUSSION

Our study once again emphasizes the extreme urgency of the problem of DGF. An expansion of criteria for deceased donation following the disproportionate increase in transplant demand will inevitably lead to higher incidence of this complication. Nevertheless, as

mentioned above, it is no secret that the DGF problem is multifactorial in nature. Our previous studies identified the main risk factors of DGF under which we proposed a set of preventive measures aimed at improving graft function in the early postoperative period.

The classical dialysis-based definition of DGF has been criticized by many authors [14]. Indeed, a number of factors may lead to the need for RRT in the first week after surgery but have nothing to do with the severity of ischemia-reperfusion injury (IRI), the morphologic basis of DGF. The most prominent examples include oligoanuria in the recipient before KT, acute rejection, or calcineurin inhibitor nephrotoxicity occurring immediately after KT. Nevertheless, a better definition has not been presented to date. In our opinion, the classical definition represents a standard that is convenient for statistical processing and subsequent analysis of outcomes, but it requires refinement.

To date, there is no unequivocal answer in the world literature as to whether the presence of DGF affects long-term survival. In an attempt to justify the expansion of criteria for deceased kidney donation, some authors claim comparable long-term survival regardless of initial function. Others, on the contrary, demonstrate increased risks of complications and graft loss in the presence of DGF.

In an attempt to clarify these contradictions, we once again retrospectively analyzed our own renal transplant

Table

Immediate kidney transplant outcomes depending on DGF

Indicator	Immediate function (A) (n = 233)	Mild DGF (B) (n = 54)	Severe DGF (C) (n = 25)	p
Recipient's age (years)	44 (IQR: 32–58)	45 (IQR: 40–52)	49 (IQR: 44–59)	0.14
Recipient's BMI (kg/m^2)	25 (IQR: 22.5–28)	26 (IQR: 24–28)	26 (IQR: 23.7–30.5)	0.51
Cold preservation time (minutes)	680 (IQR: 570–820)	710 (IQR: 670–850)	820 (IQR: 721–900)	A-C < 0.001 B-C < 0.001
Donor age (years)	47 (IQR: 38–56)	46 (IQR: 40–52)	57 (IQR: 48–59)	A-C = 0.018 B-C = 0.035
Donor BMI (kg/m^2)	26 (IQR: 24–29)	27.8 (IQR: 25–31)	31 (IQR: 26–33)	A-C = 0.032 B-C = 0.044
Median duration of DGF	0	7 (IQR: 3–9)	25 (IQR: 19–35)	A-C < 0.001 B-C < 0.001
Highest tacrolimus trough levels (C_0) in the first 7 days	12.4 (IQR: 9.2–13.4)	22.2 (IQR: 16.2–24.4)	20.6 (IQR: 15.2–26.4)	A-B = 0.03 A-C = 0.014
Rate of postoperative complications	43/233 (18.4%)	15/54 (27.8%)	14/25 (56%)	A-C = 0.02 B-C = 0.017

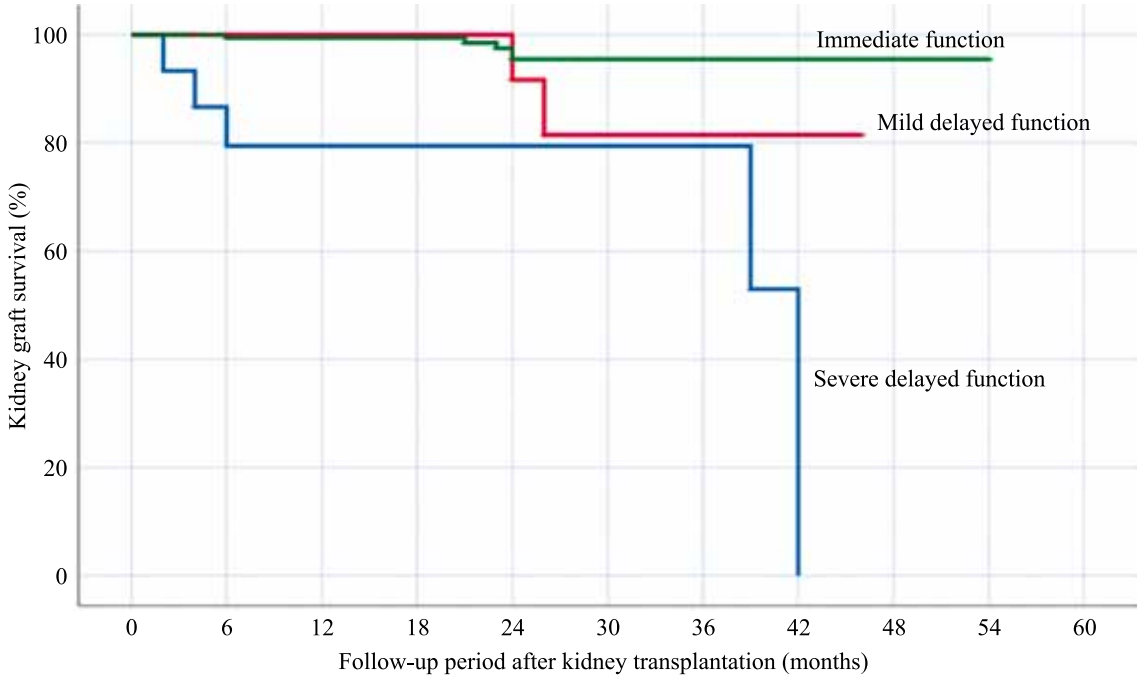

Fig. Long-term survival of kidney grafts depending on initial function

outcomes. In our opinion, DGF duration could be a clarifying indicator that can at least indirectly separate truly severe IRI from the transient need to put the recipient on dialysis. Indeed, DGF >2 weeks had a statistically significant association with postoperative complications ($p = 0.047$), as well as with lower long-term graft survival (log-rank $p = 0.021$). In our opinion, severe DGF is mainly influenced by donor characteristics, timing and peculiarities of graft preservation. Nevertheless, other factors, such as acute calcineurin inhibitor nephrotoxicity, should not be ignored. While their presence in KT from a standard donor is unlikely to lead to long-term DGF, in KT from an expanded criteria donor, they may significantly exacerbate this complication and sometimes lead to irreversible injury to the transplanted organ. Thus, prevention of all potentially modifiable risk factors for DGF should go hand in hand with expansion of donor criteria.

The authors declare no conflict of interest.

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TREATMENT OF ISCHEMIC HEART DISEASE IN END-STAGE KIDNEY DISEASE PATIENTS ON RENAL REPLACEMENT THERAPY

Yu.V. Semenova, B.L. Mironkov, Ya.L. Poz

Shumakov National Medical Research Center of Transplantology and Artificial Organs, Moscow, Russian Federation

This review paper aims to analyze the problem of diagnosis and treatment of coronary heart disease (CHD), also called ischemic heart disease (IHD), in patients with end-stage renal disease (ESRD). The analysis is based on current literature data. The issues of CHD risk stratification before patient listing for kidney transplantation (KT) and possible difficulties of diagnosing CHD using non-invasive examination methods in ESRD patients are considered. The effectiveness of myocardial revascularization and drug therapy, endovascular and surgical myocardial revascularization, is compared. The paper also discusses the peculiarities of drug therapy, particularly antiplatelet and antihyperlipidemic therapy in the treatment of CHD in dialysis-dependent patients and kidney recipients.

Keywords: end-stage renal disease, kidney transplantation, coronary heart disease, ischemic heart disease, myocardial revascularization, coronary stenting, coronary artery bypass surgery, drug therapy.

INTRODUCTION

In the last decade, there has been a rapid rise in chronic kidney disease (CKD) cases, associated with the aging population, spread of obesity, diabetes, and high blood pressure. CKD patients at all stages of the disease are characterized by a high level of cardiovascular pathology, accompanied by adverse outcomes [1]. Reports indicate that the risk of CHD is high in the early stages of CKD [2]. Renal replacement therapy (RRT) aimed at treating ESRD patients includes chronic (long-term) hemodialysis, peritoneal dialysis, and KT. KT is the gold standard treatment for ESRD. Compared to continuous RRT, successful KT offers better survival and a higher quality of life. According to the European Renal Association Registry Annual Report 2021, the life expectancy of kidney recipients is almost twice that of patients on long-term dialysis [3]. Nevertheless, cardiovascular diseases (CVD) remain one of the leading causes of mortality in kidney recipients with a functioning transplant [4].

This paper is devoted to analyzing the problems of diagnosis and treatment of CHD in patients with ESRD before and after KT, based on current literature data.

CHD RISK STRATIFICATION IN DIALYSIS-DEPENDENT PATIENTS

The goals of pre-transplant cardiovascular risk stratification are to identify asymptomatic coronary heart disease and silent ischemia, to identify surgically and anesthesiologically suitable potential kidney recipients, and to exclude patients with significant cardiovascular conditions that may lead to life-threatening perioperative complications [5]. Cardiovascular screening includes a combination of medical history, physical examination, assessment of functional status and the outcomes of non-invasive and invasive examination methods [6].

According to the 2020 Kidney Disease: Improving Global Outcomes (KDIGO) Clinical Practice Guideline on the Evaluation and Management of Candidates for Kidney Transplantation, KT candidates with symptomatic cardiac disease (e.g., angina, arrhythmia, heart failure, valvular heart disease) should be treated by a cardiologist based on current cardiac clinical guidelines. Asymptomatic kidney transplant candidates at high risk for CHD (family history of diabetes, previous coronary artery disease) or low exercise tolerance, and patients on RRT for longer than 2 years, should undergo non-invasive coronary artery disease (CAD) screening to rule out CHD. The guidelines suggest that asymptomatic patients with severe triple vessel CAD should be excluded from the KT waiting list, unless the patient has a high expected likelihood of survival after surgery [7].

Karthikeyan et al. proposed an algorithm for cardiovascular risk stratification for KT candidates. According to the algorithm, coronary angiography (CAG) is recommended for patients who have clinical signs of angina, signs of heart failure, ventricular arrhythmias, and significant pathology of the heart valve apparatus. In their absence, the authors recommended assessment of cardiovascular risk criteria: major (age ≥50 years, history of CHD and previous myocardial infarction (MI), smoking, diabetes, pulmonary embolism, hypertension, and dyslipidemia) and minor (high-density lipoprotein <0.91 mmol/L and electrocardiographic signs of left ventricular hypertrophy). If more than one major risk criterion is present, stress myocardial perfusion scinti-

Corresponding author: Yulia Semenova. Address: 1, Shchukinskaya str., Moscow, 123182, Russian Federation. Phone: (985) 250-72-82. E-mail: julie11.89@mail.ru

graphy (MPS) is indicated to decide whether CAG would be required. In the absence of major criteria, the presence of less than 2 minor criteria and good exercise tolerance (>4 Mets), patients are listed for KT, and in the case of low exercise tolerance (<4 Mets), stress MPS is performed with further decision on whether to initiate CAG [8].

Hakeem et al. proposed their coronary screening and risk stratification algorithm for ESRD patients. In their proposal, the presence and severity of clinical symptoms and echocardiographic changes (primarily impaired contractility) are assessed first, and having a history of diabetes and CHD is taken into account. Then, depending on the results, either blood troponin T levels and coronary calcium levels are assessed, or a stress test is performed. Based on the results of this test, the need to conduct CAG is decided [9].

Nimmo et al. studied 2,572 kidney recipients who received either a stress test or CAG as pre-transplant screening and compared the association of these test results with major adverse cardiac events (MACE) within 5 years after transplantation. The incidence of MACE at 90 days, 1 year, and 5 years after KT was 0.9%, 2.1%, and 9.4% respectively. There was no statistically significant association between pre-transplant screening method (stress test or CAG) and MACE at all follow-up stages. Age, male sex and history of CHD were associated with MACE [10].

Over 50% of cardiovascular mortality in ESRD patients is associated with life-threatening cardiac arrhythmias due to systolic and diastolic dysfunction, left ventricular hypertrophy, myocardial fibrosis and electric myocardial inhomogeneity, coronary calcinosis, electrolyte imbalance and hypervolemia [11]. Thus, pre-transplant screening should be aimed not only at detecting coronary atherosclerosis, but also at assessing cardiovascular risk comprehensively prior to placing patients on the KT waitlist.

In their recent study, Vadala et al. compared pre-KT cardiovascular screening algorithms proposed by major scientific societies [12]: the European Renal Best Practice Transplantation Guideline Development Group (ERBP) [13], the American Heart Association (AHA) [14, 15], and the European Society of Cardiology (ESC) [16] (Table 1).

Table 1

Algorithm for cardiovascular screening before kidney transplantation [12] according to ERBP [13], ACC 2012 [14], 2022 [15], ESC [16]

<table>
<tr><td colspan="2">Criteria for high cardiovascular risk</td><td colspan="5">• Diabetes [13–16]
• Age >60–65 years [13–16]
• Smoking [14–16]
• History of CVD [13, 14]
• Duration of dialysis >1 year [14] to 5 years [15]
• Hypertension [14, 16]
• Dyslipidemia [14, 16]
• Left ventricular hypertrophy [14]
• History of cerebrovascular disease [15]
• Peripheral atherosclerosis [15]
• History of kidney transplantation (performed >5 years ago) [15]
• Family history of CVD [16]</td></tr>
<tr><td colspan="7">Pre- kidney transplant screening</td></tr>
<tr><td colspan="2">For low-risk patients</td><td colspan="3">For high-risk patients</td><td colspan="2">For patients with a history of CHD</td></tr>
<tr><td colspan="2" rowspan="2">• Medical history [16]
• Physical examination [16]
• Standard laboratory diagnosis [16]
• Electrocardiography (ECG) [13–15]
• Echocardiography (Echo) [14, 15]
• Chest X-ray [13]</td><td colspan="3" rowspan="2">• Medical history [16]
• Physical examination [16]
• Standard laboratory diagnosis [16]
• ECG [13–16]
• Echo [13–16]
• Chest X-ray [13]
• Stress test [13–16]
• Biomarkers (troponins I, T, NT-proBNP) [16]</td><td colspan="2">Examination same as for high-risk patients</td></tr>
<tr><td>• Last CAG was >2 years ago
• Revascularization [15]</td><td>• Last CAG was <2 years ago
• No revascularization [15]</td></tr>
<tr><td colspan="2" rowspan="2">No pathology detected</td><td colspan="2">Pathology detected</td><td colspan="2" rowspan="2">Stress Echo or noninvasive assessment of myocardial perfusion [15]</td><td rowspan="4">No additional examination required [15]</td></tr>
<tr><td colspan="2">No pathology detected</td></tr>
<tr><td rowspan="2">No pathology detected</td><td rowspan="2">Pathology detected</td><td colspan="2" rowspan="2">No additional examination required [13–16]</td><td colspan="2">Stress Echo or noninvasive assessment of myocardial perfusion [13, 16]</td><td rowspan="2">No pathology detected</td><td rowspan="2">Pathology detected by examination or by previous CAG [15]</td></tr>
<tr><td>No pathology detected</td><td>Pathology detected</td></tr>
<tr><td>No additional examination required [13–16]</td><td>Stress test or CAG [13–16]</td><td>No additional examination required [13, 16]</td><td>CAG [13–16]</td><td>No additional examination required [15]</td><td>CAG [15]</td></tr>
</table>

It should be taken into account that CHD diagnosis in ESRD patients is often difficult due to insufficient information content of some tests in this category of patients, which may lead to underestimation of cardiovascular risk. Table 2 presents possible reasons for the reduced informativity of some noninvasive CHD diagnosis methods for ESRD patients [17].

TREATMENT TACTICS FOR CHD IN DIALYSIS-DEPENDENT PATIENTS

Comparison of myocardial revascularization and medical treatment

The 2020 KDIGO Clinical Practice Guideline on the Evaluation and Management of Candidates for Kidney Transplantation do not recommend MR for asymptomatic kidney transplant candidates solely for the purpose of reducing perioperative risk of cardiovascular events [7].

According to various sources, there are differences of opinion regarding the optimal treatment for CHD in KT candidates, either conservative therapy or MR.

A recent meta-analysis of 8 studies comprising 945 patients demonstrates that revascularization is not superior to optimal medical therapy (MT) in reducing all-cause mortality (RR, 1.16, 95% CI 0.63–2.12) and cardiovascular mortality (RR, 0.75, 95% CI 0.29–1.89) or MACE (RR, 0.78, 95% CI 0.30–2.07) in patients waitlisted for KT [18].

A meta-analysis of 6 studies comprising 260 kidney transplant candidates receiving medical treatment for CHD and 338 patients undergoing coronary revascularization demonstrated similar results. The analysis showed no significant differences in cardiovascular disease outcomes between the two groups (RR, 1.415, 95% CI 0.885–2.263) [19].

At the same time, several studies have shown coronary revascularization to have better outcomes than a selection of optimal MT in ESRD patients.

In 2022, a meta-analysis of 13 studies comprising 20,688 CKD patients, including dialysis-dependent patients, and patients with severe stenotic CAD was conducted. CHD was treated with either conservative therapy or MR by coronary artery stenting (CAS) or coronary artery bypass graft (CABG). The revascularization group showed lower long-term mortality (with at least a 1-year follow-up) than the conservative therapy group: both after CAS (RR 0.66, CI 0.60–0.72) and after CABG (RR 0.62, CI 0.46–0.84), including in the dialysis-dependent patient group (RR 0.68, CI 0.59–0.79) [20].

A meta-analysis of 8 studies with 1,685 dialysis-dependent patients with CAD, of whom 739 patients underwent coronary revascularization and 946 received optimal MT, showed that revascularization (RR, 0.72, 95% CI 0.62–0.84) demonstrated a significantly lower long-term all-cause mortality compared to MT. Surgical revascularization showed no significant advantage over MT in reducing all-cause mortality (RR, 0.91, 95% CI 0.57–1.46) [21].

Comparison of endovascular and surgical myocardial revascularization

Due to the development of multivessel diffuse CAD in patients with CKD, together with severe calcinosis, many authors are wondering what the optimal surgical treatment method for coronary pathology in this patient cohort could be.

A 2021 meta-analysis of 32 studies with 84,498 patients demonstrated a comparison between 3 types of CHD treatment in patients with stage 4–5 CKD: MT, CAS, and CABG. The analysis revealed that all-cause mortality was lower in the CAS group than in the MT group at different follow-up periods: ≤1 month, 1 month

Table 2

Possible reasons for the reduced informativeness of some non-invasive CHD diagnosing methods in patients with ESRD [17]

Screening tests	Limitations of study
Exercise stress test	• Baseline ECG changes • Low exercise tolerance • Hypertensive response to exercise • Insufficient chronotropic response due to autonomic dysfunction
Stress echocardiography	• Operator dependent • Narrow ultrasound window in 20% of cases • Low exercise tolerance • Hypertensive response to exercise
Myocardial perfusion scintigraphy	Low sensitivity due to: • Uniform diffuse decrease in coronary blood flow (“balanced ischemia”) • Impaired vasoreactive response
CT coronary calcium scan	• Only low coronary calcium level is of value in predicting a negative outcome
Contrast-enhanced multislice coronary CT scan	• Low specificity due to severe coronary calcification

to 3 years, and >3 years. CABG compared with conservative therapy showed no significant advantage in reducing total mortality at any of the follow-up periods. Compared to CAS, CABG demonstrated a higher risk of mortality in early postoperative periods (≤1 month) and better outcomes in long-term follow-up (1 month to 3 years and more than 3 years) due to a lower risk of cardiovascular mortality and MACEs, as well as repeat revascularization [22].

Another study featuring 112 dialysis-dependent patients who underwent CAS (n = 86) or CABG (n = 26) between 2007 and 2017, also showed a higher risk of death in patients in the CABG group in the early postoperative period (within 1 month after surgery). However, long-term outcomes (overall mortality, MACE, repeat revascularizations) did not differ between the groups [23].

Medical treatment

According to the KDIGO guidelines, kidney recipients should take aspirin, beta-blockers and statins in accordance with cardiac clinical guidelines both while on the KT waiting list and postoperatively [7].

Antiplatelet therapy has been shown to reduce cardiovascular risk in CHD patients, but the prognostic effect of this group of drugs is not so obvious in ESRD patients. A number of studies have claimed that antiplatelet agents have no significant effect when used as both primary and secondary prophylaxis of cardiovascular events and on overall mortality in dialysis-dependent patients [24, 25].

As for the management of dialysis-dependent patients after coronary stenting, according to the literature, clopidogrel is preferred as the second antiplatelet drug (from the group of P2Y12 inhibitors) in addition to aspirin because of its greater safety in this category of patients compared to newer antiplatelet agents from this group (ticagrelor, prasugrel) [26]. The use of new P2Y12 inhibitors is acceptable only in cases of high ischemic and moderate hemorrhagic risk in ESRD patients [26] or in patients with clopidogrel resistance [27].

There is controversy regarding the appropriate duration of dual antiplatelet therapy (DAPT) in dialysis patients after coronary stenting.

Some reports argue that not all ESRD patients need 12 months of DAPT after stenting – a shorter dosage regimen is acceptable for some patients [28]. The 2019 EOC Guidelines for the diagnosis and management of chronic coronary syndromes suggested a 6-month DAPT regimen with aspirin and clopidogrel after intervention, with a possible shortening of the DAPT duration to 1–3 months for patients at high hemorrhagic risk [29]. Other studies have supported the use of a 6-month DAPT in the management of ESRD patients after coronary artery stenting [30, 31].

Other authors argue for the need to use prolonged DAPT in dialysis-dependent patients – longer than the established 12 months after intervention (15, 18 months) – due to lower cardiovascular risk without a significant increase in hemorrhagic risk [32].

The European Society of Cardiology proposed the use of prolonged DAPT for secondary prophylaxis in patients at high and very high risk of ischemic events (diffuse multivessel CAD, diabetes, recurrent MI, multifocal atherosclerosis, decreased left ventricular contractility, CKD with an estimated glomerular filtration rate (eGFR) of 15–59 ml/min/1.73 m^2) and low risk of hemorrhagic events (no history of ischemic or hemorrhagic stroke, gastrointestinal bleeding, gastrointestinal pathology associated with increased bleeding risk, liver failure, coagulopathy, extreme old age or frailty, ESRD with eGFR <15 ml/min/1.73 m^2) [29].

Other studies have suggested that the size and complexity of the coronary intervention procedure itself should be considered when deciding on DAPT duration. The following factors have been proposed, in which prolonged DAPT was associated with a reduced risk of cardiovascular events: 3 coronary arteries treated, ≥3 stents implanted, ≥3 lesions treated, bifurcation with 2 stents implanted, total stent length >60 mm, recanalization of chronic occlusion, stent diameter <3 mm [33]. Coronary artery stenting in ESRD patients for acute MI was also mentioned among the indications for prolonged DAPT [34].

The effect of lipid-lowering therapy on cardiovascular risk depends on the CKD stage. Studies analyzing the effect of statin and ezetimibe therapy on cardiovascular outcomes in CKD patients, including dialysis-dependent patients, have shown that the effect of antihyperlipidemic drugs on outcomes is lower in patients with reduced eGFR and limited or absent in ESRD patients receiving dialysis treatment [35, 36]. Regarding statin dosage, it is recommended to use standard doses for CKD stages 1–2, and to reduce the dosage in advanced stages of the disease. Atorvastatin, which is practically not excreted by the kidneys but is mainly excreted by bile, is proposed as the drug of choice [36].

Hypertriglyceridemia can be managed through lifestyle modifications, including dietary adjustments, weight loss, increased physical activity, adequate glycemic control, and limitation of alcohol consumption [37].

Clinical guidelines for the management of patients with CKD K/DOQI and KDIGO do not recommend routine statins and a statins/ezetimibe combination in dialysis-dependent patients and children with CKD. However, statin or statin/ezetimibe therapy is recommended for primary and secondary prophylaxis of CVD in CKD patients not receiving RRT, as well as in patients after KT [37, 38].

The EOC and AHA guidelines recommend that statins or a statins/ezetimibe combination should be prescribed for patients with CKD stages 3–5, who are not receiving RRT, as well as for patients who, at the time of RRT initiation, were already receiving statins, ezetimibe,

or a combination of both, especially for patients with confirmed CAD. Statin therapy is not recommended in dialysis-dependent patients without confirmed coronary pathology [39, 40].

TREATMENT TACTICS FOR CHD IN KIDNEY RECIPIENTS

Comparison between myocardial revascularization and medical therapy

According to the available literature, studies comparing the treatment of CHD by coronary revascularization and selection of optimal drug therapy among post-kidney transplant patients have been less frequent than among kidney transplant candidates. The number of published reports on CABG in post-kidney transplant patients is limited [41].

A study including 1,460 kidney recipients found that correction of significant stenotic coronary lesion by coronary artery stenting (RR, 3.792, 95% CI 1.320–10.895) or CABG (RR, 6.691, 95% CI 1.200–37.323) was associated with better long-term (5-year) survival than medical therapy and was not associated with graft dysfunction and rejection [42].

Comparison of endovascular and surgical myocardial revascularization

MR techniques were compared among kidney recipients with coronary artery disease.

A recently published systematic review of 4 studies, in which 6,674 patients underwent CAS after KT and 4,402 patients underwent CABG, showed, that CAS compared with CABG was significantly associated with lower in-hospital mortality (OR 0.62, 95% CI 0.51–0.75) and 1-year postoperative mortality (OR 0.81, 95% CI 0.68–0.97), and lower acute kidney injury (AKI) prevalence (OR 0.33, 95% CI 0.13–0.84). Long-term outcomes (2–4 years of follow-up according to different studies included in the meta-analysis) were not significantly different between patients in the two groups (OR 1.05, 95%DI 0.93–1.18) [43].

A small retrospective study of kidney recipients with CHD who underwent MR by CAS (n = 27) or CABG (n = 24) showed better outcomes in the CAS group, but no significant difference between the groups was obtained: in-hospital mortality was 11.1% in the CAS group and 20.8% in the CABG group ($p = 0.45$), 1-year survival was 85.2% in the CAS group and 75% in the CABG group ($p = 0.97$), 4-year survival was 66.5% in the CAS group and 70% in the CABG group ($p = 0.97$). AKI after surgery was significantly more frequent after CABG (58.3% vs. 18.5%, $p < 0.01$). Graft survival at 1 year (95.7% in the CAS group and 94.1% in the CABG group) and at 4 years (76.8% in the CAS group and 77% in the CABG group) after revascularization was comparable between the groups ($p = 0.78$) [44].

Russian studies have confirmed the efficacy of MR by CAS after KT and its safety, manifested, among other things, by the absence of a significant negative effect of X-ray contrast agent on kidney graft function [45].

Medical treatment

The KDIGO clinical guidelines recommend diagnosing and treating CHD in post-KT patients according to the standards for the management of CHD in the general population [46].

Treatment of dyslipidemia in post-KT patients is similar to that in patients with CKD. The 2018 AHA guidelines [40] and 2019 EOC guidelines [39] recommend that kidney transplant recipients be categorized as high or very high cardiovascular risk, especially when low-density lipoprotein (LDL) levels >1.8 mmol/L, and that statins and ezetimibe should be used as first and second choice drugs for antihyperlipidemic therapy, respectively.

Statins have no proven protective effect on graft and patient survival. Nevertheless, a multicenter double-blind ALERT study, which analyzed 2,102 kidney recipients, showed a 32% reduction in LDL levels, as well as a decrease in the incidence of cardiovascular mortality and non-fatal MI in the group of patients treated with fluvastatin. At the same time, no significant difference in total mortality in the main and control groups was observed [47]. The best effect of CVD risk reduction was demonstrated when statin treatment is initiated within the first 2 years after KT [48]. Thus, a number of studies have shown that statin therapy should be recommended for kidney recipients with a well-functioning graft and an increased risk of CVD [37, 39].

Drug interactions are a common problem for post-transplant patients due to polypharmacy. Statins are metabolized in the liver by cytochrome P450, predominantly the CYP3A4 subtype. Fluvastatin, pravastatin, pitavastatin, and rosuvastatin are metabolized involving other cytochromes and are less likely to enter into drug interactions. Most statins are lipophilic except for the hydrophilic pravastatin and rosuvastatin, and therefore their use is considered safer [49]. According to the 2019 EOC guidelines [39] and 2013 KDIGO guidelines [37], it is recommended to start statin therapy at low doses with careful titration to avoid severe myopathy, rhabdomyolysis due to possible drug interactions, especially for patients receiving cyclosporine [39]. Drug interactions with tacrolimus are less frequent and dangerous compared to cyclosporine [50].

Ezetimibe is the drug of second choice for the treatment of dyslipidemia and is able to reduce LDL levels by 13–20% [49]. The American and European Societies of Cardiology recommend the use of ezetimibe in combination with statins for patients at high and very high risk of CVD or as secondary prevention to achieve target LDL cholesterol values [39, 40]. Ezetimibe can also be

prescribed as an alternative to statins in case of intolerance to statins. The use of ezetimibe with maximally tolerated statin doses has been shown to reduce dyslipidemia severity in kidney recipients without significant adverse effects on creatine phosphokinase levels and graft function [51].

The use of antihyperlipidemic drugs from other groups is limited in post-KT patients [49].

The use of aspirin in post-KT patients was analyzed in a FAVORIT trial, which showed no benefit of aspirin as primary prevention of cardiovascular events in kidney transplant recipients [52]. KDIGO clinical guidelines recommend the use of aspirin in kidney recipients with diabetes or as secondary prophylaxis in patients with confirmed CHD [46].

CONCLUSION

Pre-transplant screening for CHD in ESRD patients should not only focus on detection of coronary atherosclerosis, but also on assessing cardiovascular risk comprehensively before deciding whether to place patients on the KT waiting list.

Based on reports, endovascular MR has shown no worse, but in many cases better outcomes compared to both medical treatment and surgical revascularization for both dialysis-dependent patients and kidney recipients.

Drug treatment of CHD in dialysis-dependent patients and kidney recipients is generally consistent with drug treatment of CHD in the general population, but the specifics of antiplatelet agents and statins in these patients should be considered.

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CLINICAL COURSE OF ASCITIC SYNDROME AND ACUTE KIDNEY INJURY IN THE SETTING OF NONSELECTIVE BETA-BLOCKERS OR ENDOSCOPIC VARICEAL LIGATION FOR PRIMARY PREVENTION OF BLEEDING IN CIRRHOTIC PATIENTS AWAITING LIVER TRANSPLANTATION

R.V. Korobka[1, 2], S.V. Gautier[3, 4], V.D. Pasechnikov[1, 5], E.S. Pak[1, 2], A.M. Shapovalov[1], Yu.V. Khoronko[2], D.V. Pasechnikov[5], I.A. Porshennikov[6, 7]

[1] Rostov Regional Clinical Hospital, Rostov-on-Don, Russian Federation
[2] Rostov State Medical University, Rostov-on-Don, Russian Federation
[3] Shumakov National Medical Research Center of Transplantology and Artificial Organs, Moscow, Russian Federation
[4] Sechenov University, Moscow, Russian Federation
[5] Stavropol State Medical University, Stavropol, Russian Federation
[6] Novosibirsk Regional Clinical Hospital, Novosibirsk, Russian Federation
[7] Novosibirsk State Medical University, Novosibirsk, Russian Federation

Objective: to compare the effects of nonselective beta-blockers (NSBB) and endoscopic variceal ligation (EVL) on patient survival, ascites dynamics, and development of acute kidney injury (AKI) during primary prevention of bleeding from the esophageal varices and cardia in patients with decompensated cirrhosis on the liver transplant waiting list (LTWL). **Materials and methods.** A retrospective comparative study of the clinical data of patients with severe ascites and esophageal varices without a bleeding history at the time of their inclusion in the LTWL was performed. Group 1 patients (n = 84) were prescribed NSBB, alpha and beta-adrenoblockers in order to prevent bleeding and reduce progression of decompensated cirrhosis. Group 2 patients underwent EVL. **Results.** Demographic, laboratory and instrumental parameters of patients in the compared groups had no significant differences. In both groups, there were no significant differences between the indicators of severity of liver lesions (MELD-Na, Child–Turcotte–Pugh), frequency of severe ascites, frequency of varicose nodes grades 2–3. At follow-up, bleeding developed in 22 patients (13.25%) – 13 patients in the NSBB group and 9 patients in the EVL group (15.47% and 10.97%, respectively, $p > 0.05$). Patient survival was significantly higher in the EVL group than in the NSBB group. Incidence of refractory ascites, number of patients with grade 3 ascites, and AKI stages 2–3 in the NSBB group, were significantly higher ($p < 0.05$) than in the EVL group. MELD-Na was the independent predictor of mortality in the EVL group, while low mean arterial pressure (mAP) and presence of AKI were those for patients receiving NSBB. **Conclusion.** NSBB and EVL are effective methods of primary prevention of bleeding. Mortality rate, number of patients with refractory ascites and severe ascites, and number of patients with AKI stages 2–3 were higher in the NSBB group than in the EVL cohort. In EVL patients, the independent predictor of death was MELD-Na, while in NSBB patients, the independent predictors of mortality were low mAP and presence of AKI.

Keywords: liver transplant waiting list, ascites, variceal bleeding, endoscopic variceal ligation, nonselective beta blockers, acute kidney injury, MELD-Na, mean arterial pressure.

INTRODUCTION

NSBB and EVL are means of curbing the progression of decompensated cirrhosis after occurrence of the first decompensating event, most often ascites [1, 2]. The term "progression of decompensated cirrhosis" was introduced into clinical practice by the International Consensus on the Diagnosis, Treatment and Prevention of Cirrhosis Complications (Baveno VII) [3]. According to the authors of the Consensus, the term "progression of decompensated cirrhosis" implies the presence of a prognostic stage characterized by a higher patient mortality than in the first episode of decompensation [3]. Several factors are considered as drivers of progression of decompensated cirrhosis: variceal bleeding (VB) or gastric bleeding (GB), diuretic-resistant ascites or a significant increase in the clinical severity of ascites, manifestations of hepatic

Corresponding author: Victor Pasechnikov. Address: 21, Aviatsionnaya str., Stavropol, 355017, Russian Federation. Phone: (962) 447-75-13. E-mail: passetchnikov@mail.ru

encephalopathy (HE) [3]. Measures to prevent progression of decompensated cirrhosis include prophylaxis of the first bleeding episode in patients with varices at low or high risk of VB or GB. Baveno VII experts prioritize traditional NSBB or carvedilol. In cases of intolerance or contraindications to the use of this class of drugs, an interventional procedure – EVL – is recommended. Despite the relative effectiveness of primary prevention of bleeding in patients with ascites, the first episode of decompensated cirrhosis is an indication for inclusion in the LTWL [3]. In all transplant systems in Europe, USA, Russia, etc. there is a gap between the number of LTWL patients and the number of LT performed. This is proportionally related to increased decompensated cirrhosis and, accordingly, indications for LT [1, 3] on one hand, and organ shortages [4–6] on the other hand. Increased LTWL time causes further decompensation due to the risk of recurrent events (bleeding, diuretic-resistant ascites, development of manifest HE, etc.). In this regard, therapeutic measures aimed at preventing further decompensation and, accordingly, at preserving the life of this group of patients, are extremely relevant [3, 7].

Ascites is the most common decompensating event in cirrhosis, and it is associated with high morbidity and mortality rates [8, 9]. After the development of ascites, further decompensating events in cirrhosis may develop, which are subclassified as ascites-related (spontaneous bacterial peritonitis, dilutional hyponatremia and acute liver injury [10–12]), or ascites-unrelated (VB and HE [13]), which complicate the clinical course of the disease [9, 13].

MATERIALS AND METHODS

The comparative retrospective study included 166 cases with decompensated cirrhosis who were included in the LTWL between 2016 and 2022.

Inclusion criteria: ascites of varying severity, no variceal bleeding prior to inclusion in the LTWL, abstinence for at least 3 months (confirmed by addiction specialists) prior to inclusion in the list for patients with alcohol-related cirrhosis, virus-related cirrhosis (hepatitis B virus (HBV)- or hepatitis C virus (HCV)-associated etiology), cirrhosis of mixed etiology (virus-related and alcohol-related), cirrhosis classes B and C according to the Child–Turcotte–Pugh (CTP) classification.

Exclusion criteria: patients with any tumors, including hepatocellular cancer, accompanied by ascites, HE grade 2 and above, any infections, portal vein thrombosis, renal dysfunction at the time of inclusion in the study, refractory ascites, contraindications to NSBB (bradyarrhythmia, bronchial asthma, obstructive pulmonary disease), and diabetes mellitus.

Group 1 included 84 patients and group 2 had 82 patients. Both groups of patients with ascites, as the first episode of the beginning phase of decompensated cirrhosis, were included in the LTWL. Patients from the first group with signs of high risk of first VB received NSBB or carvedilol for primary prophylaxis. Group 2 patients underwent EVL for the same purposes due to intolerance and/or contraindications to NSBB or carvedilol.

Concurrently, we investigated the survival rates among patients who received NSBB or underwent EVL during primary prophylaxis of bleeding in those with decompensated cirrhosis included in the LTWL (primary endpoint of the study) and determined the effect of NSBB and EVL on the dynamics of ascites and AKI during primary prevention of bleeding in patients with decompensated cirrhosis included in the LTWL (secondary endpoint of the study).

All data, including demographic, clinical, and laboratory parameters, were obtained from a permanent, continuously updated electronic database of patients who were under follow-up after their inclusion in the LTWL, after approval of the study by the Local Ethics Committee, Center for Surgery and Donation Coordination, Rostov Regional Clinical Hospital.

Clinical and biochemical blood tests, hemostasis parameters, calculation of MELD-Na scores and liver injury class according to CTP were repeated at 3-month intervals where the patients' condition was stable.

Where patients' condition was stable, abdominal ultrasound was carried out every 6 months (of waiting for LT) after the patients' initial examination.

In all patients, esophagogastroduodenoscopy (EGD) was performed to screen for varices with high risk of VB. The Baveno VI [14] and World Gastroenterology Association (WGO) [15] guidelines were used to identify patients with varices requiring urgent therapy (medium and large varices).

The severity of ascites in patients included in the study was determined according to the International Ascites Club criteria [16]. To diagnose AKI, we used the criteria proposed by the experts of the International Kidney Disease Improving Global Outcomes (Kidney Disease Improving Global Outcomes), modified by experts from the International Ascites Club [17, 18].

Mean arterial pressure (mAP) was determined by the formula: $mAP = (DP) + {}^{1}/_{3}(SP - DP)$, where SP is systolic pressure, and DP is diastolic pressure [19].

In order to prevent "further decompensation", we performed primary prophylaxis of VB using the traditional NSBB (propranolol, nadolol) and carvedilol. Propranolol was initiated at a starting dose of 40 mg/day, with a maximum dose of 240 mg/day; nadolol was 40 mg/day and 80 mg/day, respectively. The starting dose of carvedilol was 6.25 mg/day and the maximum dose was 25 mg/day. Heart rate, SP, DP and mAP were monitored in all patients using these drugs. Drug doses were adjusted whenever these parameters decreased.

A multi-band ligation kit was used to perform EVL. EGD was performed under sedation for this purpose.

EVL began at the gastroesophageal junction and proceeded proximally. Typically, 2 to 4 rubber ligatures were used depending on the size of varices. A repeat EVL was performed 4 weeks after the first, and subsequent EVLs were repeated until all varices, subject to emergency treatment criteria [14, 15], were obliterated. After achieving the result (obliteration of esophageal varices), control EGDs were performed at 3-month intervals. Where there are recurrences (appearance of new varices), repeat EVL was performed.

Patients in both groups received diuretics; paracentesis was performed in patients with diuretic-resistant ascites. Patients with AKI stage 2–3 were considered as a priority group for priority LT. During the waiting period for LT, patients with AKI stage 2–3 were discontinued from diuretics and received intravenous infusions of albumin and terlipressin.

According to the guidelines for the treatment of patients with HBV- and HCV-associated cirrhosis who are waitlisted for LT, antiviral therapy with nucleoside alternatives and a combination of direct-acting antivirals was performed, respectively [20].

The obtained data were analyzed using IBM SPSS Statistics (version 23). The type of distribution of the obtained variables of sample indicators (normal and non-normal distribution) was determined by calculating the Kolmogorov–Smirnov test. In the case of normal distribution, the variables were presented as arithmetic mean (M) with determination of standard deviation (SD); significance of differences between compared values was determined by Student's t-test. In the case of non-normal distribution, variables were expressed by means of median (Me) and interquartile range (IQR, interval between the 75th and 25th percentiles of the data). To determine the significance of differences between variables, the following nonparametric criteria were used: Wilcoxon test for pairwise comparisons of dependent variables, Mann–Whitney U test and Pearson's Chi-square for comparison of independent variables. Frequency and proportion (%) analysis was used to compare qualitative parameters. The p value <0.05 was accepted as the criterion of statistical significance between compared parameters. Patient survival in the compared groups was determined by the Kaplan–Meier method. The significance of differences between compared curves was determined by calculating the logarithmic test [Log-Rank (Mantel-Cox)].

To determine the probability of an event depending on the values of independent variables (risk factors or predictors), we used a binary logistic regression method with stepwise removal of insignificant predictors by the backward elimination (Wald) method. To assess the quality of the regression model (predictive ability), the ROC (Receiver Operating Characteristic) curve was plotted and the area under the ROC curve (AUC) was calculated. The null hypothesis was that the AUC ROC curve did not differ from 0.5. The Mantel–Haenszel odds ratio (OR) was used to assess the association between the tested outcome and the risk factor, and the 95% confidence interval (CI) for this indicator was determined. A comparative assessment of accumulated risks in the groups was performed using a mathematical model of proportional risks (Cox regression). The risk of occurrence of the test event (HR, hazard ratio) was calculated with determination of 95% confidence interval (CI) for this indicator.

RESULTS

Data on demographic, clinical, laboratory parameters, indices (MELD-Na, CTP) in the groups of patients who received NSBB (n = 84) or underwent EVL (n = 82) during LTWL stay are presented in Table 1 and Table 2.

As can be seen from the tables presented, the demographic, laboratory and instrumental parameters of patients with decompensated cirrhosis in the compared groups had no significant differences. In both groups of patients included in the LTWL, there were no significant differences between the severity of liver injury represented by MELD-Na score and CTP class.

There were no significant differences in the pattern of etiology (virus-related, alcohol-related, mixed) in the compared groups of patients with decompensated cirrhosis. In patients enrolled in LTWL, grade 2 ascites prevailed without significant differences between the groups; the incidence of grade 3 ascites was also comparable in the compared groups ($p > 0.05$). Grade 2 varices prevailed in both groups without significant differences between groups ($p > 0.05$). There were also no significant differences ($p > 0.05$) in the incidence of grade 3 varices among the compared groups of patients.

During the follow-up period up to 18 months of LTWL stay, VB developed in 22 patients (13.25%) – 13 patients in NSBB group and 9 in EVL group (15.47% and 10.97%, respectively, $p > 0.05$).

During the LT wait period, 53 patients (31.92%) died in both groups: 36 patients in the NSBB-treated group and 17 patients in EVL group (42.85% and 20.73%, respectively, $p < 0.05$). Thus, patient survival was significantly higher in EVL than in NSBB group, as determined by the Kaplan–Meier method (Log Rank = 0.004) (Fig. 1).

While waiting for LT during 18 months of follow-up, both patient groups developed refractory ascites (20 patients, 10.75%). The frequency of refractory ascites in NSBB group was significantly higher ($p < 0.05$) than in EVL group (Table 3). The number of patients with grade 3 ascites and AKI stages 2–3 increased in NSBB group compared to EVL group during the mentioned LTWL stay period (Table 3).

To search for possible risk factors for death and predictors influencing mortality, a comparative analysis was performed in the groups of deceased and survivors at the time of follow-up, who received NSBB in LTWL

or underwent EVL. Using the binary logistic regression method with stepwise removal of insignificant predictors by the backward Wald exclusion method, we were able to identify significant predictors of mortality (Table 4).

As shown in Table 4, MELD-Na, CTP class, platelet and leukocyte counts were significant predictors of mortality in EVL group. To test the suitability of the regression model for predicting the risk of waitlist mortality, ROC analysis of the identified predictors was performed to obtain ROC curves and calculate the area under them (AUC) (Table 5 and Fig. 2).

From Table 5 and Fig. 2, it can be concluded that the predictors included in the regression model (MELD-Na, platelet and leukocyte counts) significantly affect waitlist

Table 1

Comparative characteristics of the indicators in the NSBB and ELV groups (normal and non-normal distribution)

Indicator	NSBB (n = 84) M ± SD	EVL (n = 82) M ± SD	Statistical significance
Normal distribution (M ± SD)			
Age	51.36 ± 11.43	49.57 ± 11.98	p > 0.05
Hemoglobin (g/L)	110.57 ± 24.18	114.57 ± 25.83	p > 0.05
Leukocytes ($\times 10^9$/L)	3.25 ± 0.67	3.19 ± 0.79	p > 0.05
Platelets ($\times 10^9$/L)	79.87 ± 32.75	75.67 ± 35.39	p > 0.05
Plasma albumin (g/L)	38.78 ± 4.67	36.23 ± 4.25	p > 0.05
MELD-Na	22.12 ± 4.57	21.49 ± 5.21	p > 0.05
mAP (mmHg)	76.35 ± 21.54	77.54 ± 24.35	p > 0.05
SP (mmHg)	111.15 ± 29.34	109.56 ± 31.05	p > 0.05
DP (mmHg)	62.21 ± 19.31	67.54 ± 18.57	p > 0.05
Non-normal distribution (Me; IQR)			
INR	2.02 (1.59–2.43)	1.90 (1.81–2.18)	p > 0.05
Bilirubin (μmol/L)	69.0 (57.5–108.5)	65.0 (53.00–105.00)	p > 0.05
Creatinine (μmol/L)	92.0 (68.55–120.5)	88.0 (63.5–119.5)	p > 0.05
Na (mmol/L)	137.5 (118.5–149.5)	134.5 (104.5–170.5)	p > 0.05

Table 2

Comparative characteristics of indicators (sex, etiology of cirrhosis, severity of ascites, severity of esophageal varices, class of cirrhosis) in the NSBB and ELV groups

Indicator	NSBB (n = 84) (%)	EVL (n = 82) (%)	Statistical significance
Male	62 (73.81%)	63 (76.83%)	p > 0.05
Virus-related cirrhosis	49 (58.33%)	47 (57.32%)	p > 0.05
Alcohol-related cirrhosis	25 (29.77%)	27 (32.92%)	p > 0.05
Cirrhosis of mixed etiology	10 (11.90%)	8 (9.76%)	p > 0.05
Ascites, grade 2	62 (73.81%)	63 (76.83%)	p > 0.05
Ascites, grade 3	22 (26.19%)	19 (23.17%)	p > 0.05
Esophageal varices, grade 2	59 (70.24%)	57 (69.51%)	p > 0.05
Esophageal varices, grade 3	25 (29.76%)	25 (30.49%)	p > 0.05
CTP class B	5 (5.95%)	7 (8.54%)	p > 0.05
CTP class C	79 (94.05%)	75 (91.46%)	p > 0.05

Table 3

Comparative characteristics of indicators in the NSBB and ELV groups 18 months since start of the study

Indicator	NSBB (n = 84) (%)	EVL (n = 82) (%)	Statistical significance
Refractory ascites	16 (19.05%)	4 (4.88%)	p < 0.05
Ascites, grade 2	24 (28.57%)	56 (68.29%)	p < 0.05
Ascites, grade 3	44 (52.38%)	22 (26.83%)	p < 0.05
AKI, stage 1	6 (7.14%)	4 (4.88%)	p > 0.05
AKI, stage 2	13 (15.48%)	2 (2.44%)	p < 0.05
AKI, stage 3	11 (13.10%)	3 (3.66%)	p < 0.05

mortality at 18 months. At the same time, MELD-Na is an independent predictor of mortality.

Since the AUC ROC values for leukocyte and platelet counts and CTP class were below 0.5, these indicators were excluded from the analysis due to their unsuitability for use as independent predictors in the mathematical model.

The Mantel–Haenszel OR for mortality in EVL group if the MELD-Na score at LTWL inclusion was >25 was 2.077 (95% CI 1.562–2.92); if MELD-Na ≤25, the OR was 0.238 (95% CI 0.155–0.365); $p < 0.0001$.

To clarify the association between NSBB, AKI and waitlist mortality, we used Cox proportional hazards mathematical regression model with calculation of risk of death (HR) and determination of 95% confidence interval (CI).

As shown in Table 6, two independent risk factors, mAP score and AKI (HR = 2.220; p = 0.001; 95% CI [0.890–5.534] and HR = 4.601; p = 0.005; 95% CI [1.747–11.163], respectively), significantly influenced mortality rates in NSBB group.

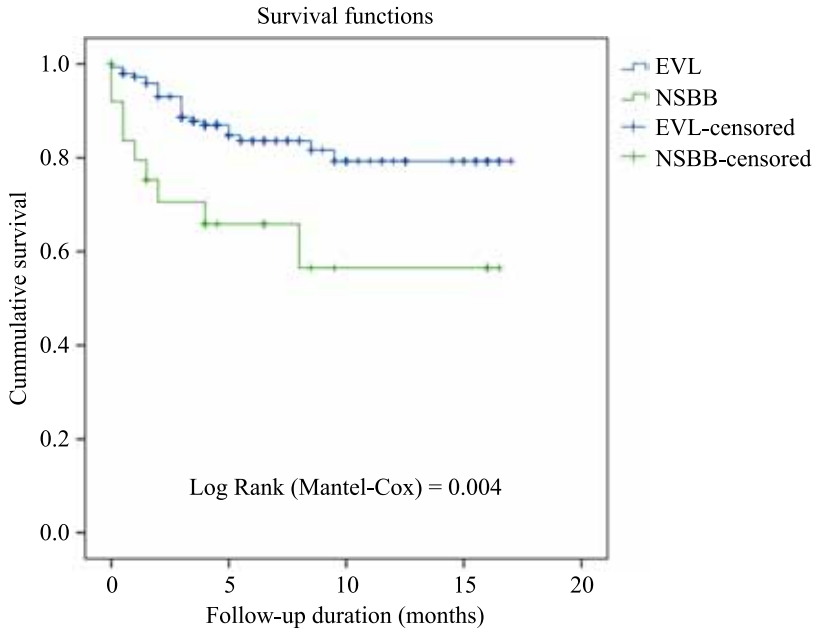

Fig. 1. Patient survival in the EVL and NSBB groups (Kaplan–Meier estimate with Log-Rank test)

Table 4

Variables in the binary logistic regression equation

Variable	B	Root mean square error	Wald	p-value	Exp (B)	95% CI for Exp (B)	
						Lower bound	Upper bound
MELD-Na	0.080	0.041	3.874	0.049	1.083	1.000	1.173
Platelets	–0.012	0.006	3.952	0.047	0.988	0.976	1.000
Leukocytes	–1.130	0.280	16.261	0.001	0.323	0.187	0.560
CTP class	1.723	0.767	5.051	0.025	5.601	1.247	25.163
Constant	1.374	1.563	0.773	0.379	3.950		

Note. Independent variables (creatinine and albumin levels) whose values were not significant (p > 0.05) are not shown in the table.

Table 5

Characteristics of the predictive value of the resulting model

Variables	Area under the curve	Standard error	Asymptotic significance	Asymptotic 95% CI	
				Lower bound	Upper bound
MELD-Na	0.737	0.042	<0.001	0.655	0.818
CTP class	0.476	0.040	0.569	0.398	0.555
Platelets	0.288	0.037	<0.001	0.214	0.361
Leukocytes	0.225	0.033	<0.001	0.160	0.289

DISCUSSION

Introduction of the main provisions of the Baveno VII Consensus into clinical practice led to a change in the way portal hypertension (PH) is treated. The main task was not to control the course (treatment) of ascites, but to prevent "progression of decompensated cirrhosis" (i.e. influence on the mechanisms of cirrhosis progression) and reduce patient mortality [3].

We found that when primary prophylaxis of bleeding in patients with decompensated cirrhosis included in the LTWL, survival was significantly higher in the group of patients who underwent EVL than in the NSBB group. This difference was due to a higher mortality rate in NSBB group compared to EVL group (42.85% and 20.73%, respectively, $p < 0.05$).

Similar results to our work were obtained in a randomized clinical trial (RCT) that included patients with severe ascites and varices requiring primary prevention of bleeding. It was shown that the survival rate of patients treated with NSBB (propranolol) was lower than in the EVL group (76.0% and 89.7%, respectively, $p = 0.02$) [21].

In another study involving two groups of patients with compensated cirrhosis (with and with no NSBB), NSBB was found to improve patient survival at up to 3 years of follow-up [22]. In particular, the use of NSBB resulted in increased survival in the group of patients awaiting LT compared to patients not receiving these drugs (HR: 0.319, 95% CI: 0.120–0.848; $p = 0.022$). However, compared with our study and the RCT cited above [21], most patients had compensated cirrhosis in this study and a relatively low MELD score (51.1%, CTP class A, MELD: 12.1 ± 3.8). In our study, CTP grade C was dominant among patients in both groups (94.05% and 91.46%, respectively), MELD scores also had a higher gradation (22.12 ± 4.57 and 21.49 ± 5.21, respectively).

In our study, both methods of primary prevention of bleeding were quite effective, as evidenced by the low incidence of VB during an 18-month follow-up period.

Previously, we obtained similar results comparing NSBB and EVL for primary prevention of VB at follow-up periods ranging from 1 month to 36 months in the LTWL [23]. Similar results to our data were obtained by Singh et al. [21]. The RCT authors found that the

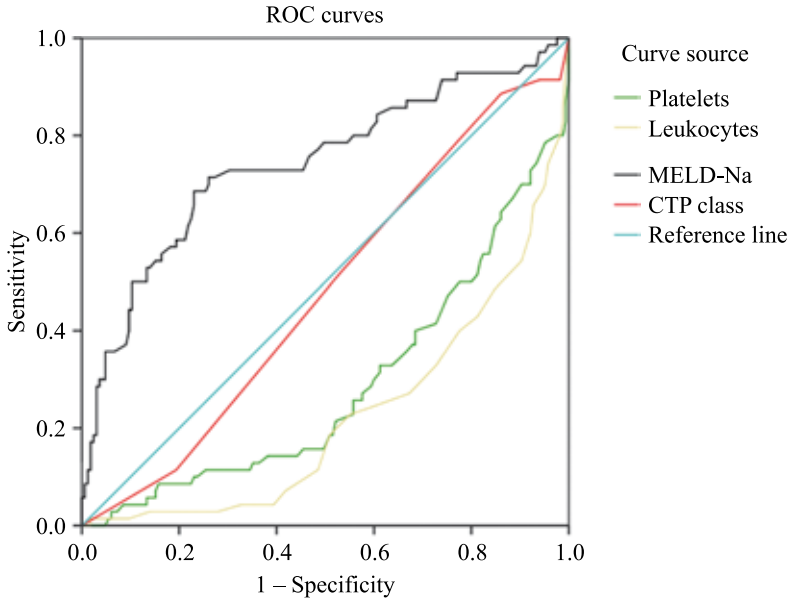

Fig. 2. ROC curves of predictors of mortality during LTWL stays of up to 18 months in EVL subjects

Table 6

Variables in the Cox regression equation (proportional hazards model)

Variable	B	Root mean square error	Wald	p-value	Exp (B)	95% CI for Exp (B)	
						Lower	Upper
Leukocytes	–0.629	0.201	9.843	0.053	0.533	0.360	0.790
Creatinine	0.005	0.004	1.630	0.202	1.005	0.997	1.012
mAP	0.797	0.032	2.926	0.001	2.220	0.890	5.534
MELD-Na	0.036	0.046	0.602	0.438	1.037	0.360	0.790
AKI	1.723	0.767	5.051	0.005	4.601	1.747	11.163

incidence of VB when comparing NSBB group and EVL group was 7.5% and 2.5%, respectively, p = 0.13.

Pérez-Ayuso et al. [24] showed no significant differences in the incidence of bleeding when comparing NSBB (propranolol) and EVL used in primary prophylaxis of VB.

Wei et al. [25] found NSBB (carvedilol) and ELV to have equal efficacy in primary prophylaxis of bleeding at 6, 12, 18 and 24 months of follow-up.

Pfisterer et al. [26] found no significant differences between the efficacy of NSBB (propranolol, carvedilol) and EVL in primary prophylaxis of VB at up to 3 years follow-up period. The study authors showed that bleeding rates at 1 year for NSBB and EVL were 7.5% and 9.9%, respectively, (p > 0.05); at 2 years, 15.5% and 16.7%, respectively (p > 0.05); and after three years, 18% and 19.7%, respectively (p > 0.05).

We found that both compared groups developed refractoriness to the current therapy for ascites, the incidence of which was significantly higher in NSBB patients than in EVL group. In addition, the proportion of patients with grade 3 ascites increased in NSBB cohort.

Singh et al. [21] also noted a significant increase in the proportion of patients with worsening ascites in NSBB (propranolol) group compared to EVL group (15% and 5%, respectively, p = 0.03), as well as an increase in the proportion of patients who developed diuretic-resistant ascites (13.7% and 3.7%; respectively, p = 0.02).

We also found that over the 18-month LTWL stay, the proportion of patients with stage 2–3 AKI significantly increased in those receiving NSBB compared to the EVL-treated group.

An increase in the proportion of patients with AKI while receiving NSBB compared to patients receiving EVL was noted in the work of Singh et al. [21]. AKI was diagnosed in 26.2% of cases for NSBB and in 12.5% of cases for EVL, p = 0.02.

Lai et al. [27] showed that the use of NSBB in patients undergoing LTWL was associated with stage 2 AKI (HR = 1.8; 95% CI 1.26–2.57) in patients with decompensated cirrhosis (CTP grade C). The authors concluded that in patients with decompensated cirrhosis awaiting LT, the use of NSBB is undesirable because it is associated with a high risk of AKI.

We found that MELD-Na score is an independent predictor of mortality in patients undergoing EVL. It was shown that the risk of mortality in this category of patients (Mantel–Haenszel OR) depends on MELD-Na score. If MELD-Na score >25, the Mantel–Haenszel OR is 2.077 (95% CI 1.562–2.92), and if MELD-Na score <25, mortality risk is significantly lower (OR = 0.238 (95% CI 0.155–0.365); p < 0.0001.

Our findings about MELD-Na as an independent predictor of LTWL mortality are confirmed by Sinh et al. [21], who obtained similar results. Lai et al. [27] found that the risk of mortality in patients with cirrhosis, CTP class, awaiting LT was associated with NSBB (HR = 1.45; 95% CI 1.03–2.03).

We showed, using a mathematical Cox proportional hazards regression model, that two independent risk factors determine the development of mortality while taking NSBB: mAP score and AKI (HR = 2.220; p = 0.001; 95% CI [0.890–5.534] and HR = 4.601; p = 0.005; 95% CI [1.747–11.163], respectively).

In addition to the MELD-Na score, mAP <82 mmHg was also considered an independent risk factor for mortality, which is supported by our study [21].

In another study, multivariate analysis showed that the presence of ascites (HR: 3.901, 95% CI: 1.352–11.251; p = 0.012) and pre-existing renal impairment (HR: 4.315, 95% CI: 1.054–17.672; p = 0.012) were independent risk factors for AKI with NSBB in a cohort of patients with cirrhosis and varices requiring therapy [22].

In a prospective study, Sersté et al. [28] showed that NSBB was associated with lower mAP compared with the group of patients who did not receive these drugs (78 ± 3 mmHg and 87 ± 5 mmHg, respectively, p < 0.0001). Among patients taking NSBB during 168 days of follow-up, 89.6% (95%CI, 74.9–95.9%) developed AKI, compared with 50.4 (95%CI: 39.0–60.7) in patients not taking NSBB; p = 0.0001). In a multivariate analysis, the authors found independent predictors of AKI: high MELD score and NSBB. Ngwa et al. concluded that patients who took NSBB were more likely to develop AKI within a 90-day period than patients who did not take these drugs (22% and 11%, respectively, p = 0.048) [29].

CONCLUSION

Receiving NSBB and performing EVL in patients with cirrhosis, varices and ascites are effective methods of primary prevention of VB.

At the same time, mortality rates in patients receiving NSBB while waiting for LT was higher than in the group of patients undergoing EVL.

In NSBB group, there was an increased number of cases of diuretic-resistant ascites compared to EVL group, and there was an increased number of patients with more severe ascites.

In addition, the proportion of patients with AKI stages 2–3 in the group of patients who received NSBB during 18 months of LTWL stay increased significantly compared to EVL group.

MELD-Na score is an independent predictor of mortality in EVL patients. The risk of mortality (Mantel–Hentzel OS) in this category of patients depends on MELD-Na score.

Two independent risk factors determine mortality rates in patients who took NSBB: low mAP and presence of AKI.

The authors declare no conflict of interest.

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FABRICATION OF A 3D PRINTED EVEROLIMUS-ELUTING STENT MADE OF THERMOPLASTIC POLYURETHANE AND POLYLACTIDE

M.T. Bekov[1], I.V. Pashkov[1], K.S. Smirnov[1], Ya.S. Yakunin[1], D.N. Shilkin[2], I.S. Chashchin[3], N.M. Ivanova[4], O.M. Tsirulnikova[1, 5], S.V. Gautier[1, 5]

[1] Shumakov National Medical Research Center of Transplantology and Artificial Organs, Moscow, Russian Federation

[2] Bakulev Scientific Center of Cardiovascular Surgery, Moscow, Russian Federation.

[3] Nesmeyanov Institute of Organoelement Compounds, Moscow, Russian Federation

[4] Zelinsky Institute of Organic Chemistry, Moscow, Russian Federation

[5] Sechenov University, Moscow, Russian Federation

Bronchial stenoses are one of the most common airways complications after lung transplantation. One of the main methods to restore airway patency is bronchial stenting. However, bronchial stenting is associated with a number of complications, such as stent migration, granulation tissue formation along the proximal and distal edges, and mucus obstruction of the lumen. This article demonstrates the possibility of manufacturing an everolimus-eluting stent from thermoplastic polyurethane and polylactide using 3D printing.

Keywords: lung transplantation, airways complications, bronchial stenosis, bronchial stenting, silicone stents, metal stents, bioresorbable stents, 3D printing, drug-eluting stents.

Bronchial stenosis is one of the most common airway complications occurring in lung transplant survivors. There is a definite pattern between the types of bronchial complications and the timing of their occurrence [1]. The most common time for stenosis occurrence is the first 2–9 months after lung transplantation [2]. Bronchial stenting is a generally accepted technique for restoring and preserving airway lumen with subsequent prevention of occurrence [3–8].

Based on fabrication material, there are usually two groups of stents used in interventional pulmonology: silicone and metallic stents [9]. Stents made of biodegradable materials should be singled out as a separate group.

There are several variations of silicone stents that differ in shape. The main types include T-tube tracheal stent [10], Dumon stent [11], Y-stents [12] (Fig. 1).

In turn, metallic stents used in endoscopic practice can be divided into self-expanding and balloon-expandable stents. In addition, stents are divided into covered and uncovered [13] (Fig. 2).

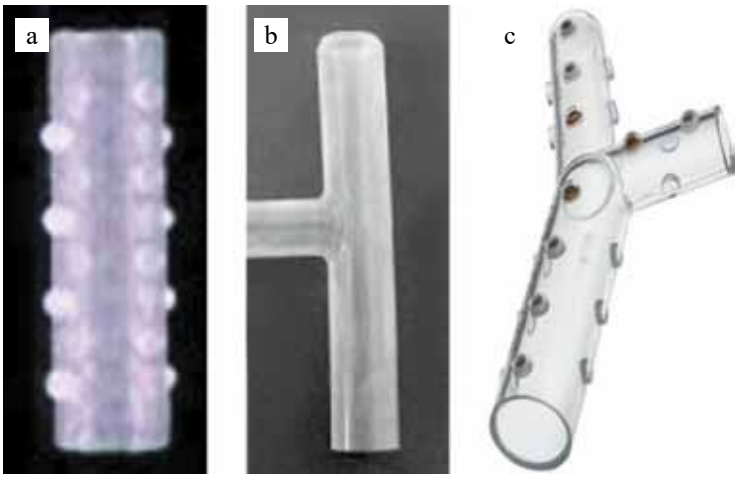

Fig. 1. Main types of silicone stents: a, Dumon stent; b, Montgomery stent; c, Oki Y-stent

Corresponding author: Maksat Bekov. Address: 1, Shcukinskaya str., Moscow, 123182, Russian Federation. Phone: (926) 399-49-75. E-mail: doctorbekov@gmail.com

MAJOR COMPLICATIONS ASSOCIATED WITH TRACHEOBRONCHIAL TREE STENTING

Each of the stent options considered has both advantages and disadvantages that determine the choice of a particular stent in a specific clinical situation.

The main complications associated with having a stent in the lumen of the tracheobronchial tree include granulation tissue growth, stent migration, and sputum obturation.

Stent migration is more common when using Dumon silicone stents than when using Y-shaped silicone or metallic stents [12]. Migration can be caused by a mismatch between the size of the stent and the airway, including the mismatch between the stent and the anatomy of the tracheobronchial tree. Stents can migrate both proximally and distally. Proximal migration can cause acute severe respiratory failure (Fig. 3).

Distal stent migration can disrupt bronchial ventilation with the development of atelectasis and pneumonia.

Stent obstruction by bronchial secretion occurs as a result of stagnation of the content, which is normally removed from the bronchial lumen. Eventually, impaired evacuation of bronchial contents may lead to stent colonization [14]. As a rule, stent obstruction is most characteristic when using long silicone or covered nitinol stents, as well as stents with complex configuration (Montgomery stent, Y-stent).

The presence of stent in the lumen of the tracheobronchial tree causes local inflammatory response, leading to the growth of granulation tissues [15] (Fig. 4).

The inflammatory response is caused by local tissue hypoxia, which activates a cascade that releases chemokines and cytokines [16]. The end result is fibroblast activation and proliferation. Granulation tissue growth occurs predominantly along the proximal and distal edges.

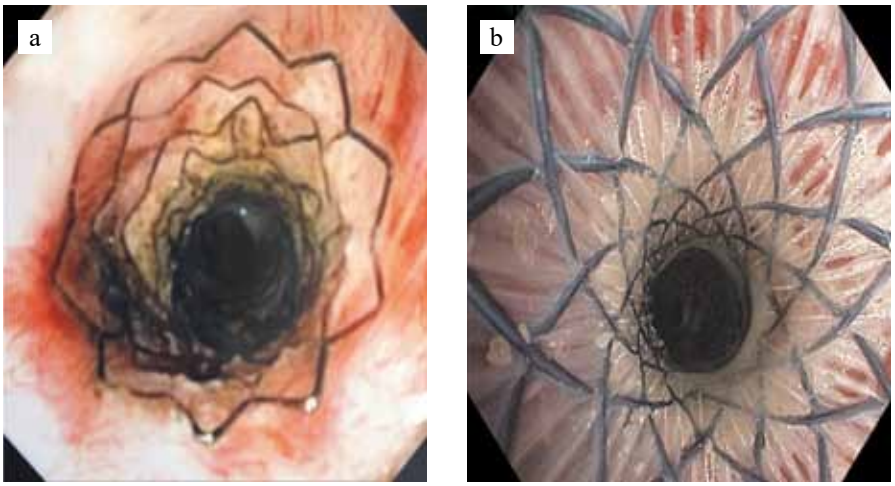

Fig. 2. Types of stents by coating: a, uncoated metallic stent; b, coated metallic stent

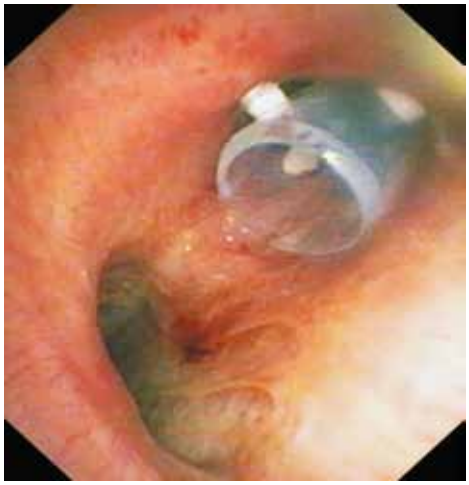

Fig. 3. Proximal migration of a stent placed in the intermediate bronchus, blocking the lumen of the right superior lobe bronchus

Uncovered metallic stents have a high rate of recurrent stenosis, including due to the sprouting of granulation tissue into the stent lumen [17]. When using covered metallic stents, the granulation tissue does not grow into the stent lumen.

BIODEGRADABLE STENTS

The use of biodegradable materials in stent fabrication should reduce tissue reactivity to foreign bodies in the airway lumen. Also, the ability of the stent to hydrolyze and its subsequent degradation may probably reduce the incidence of biofilm formation and stent colonization.

The timing of stent retention in the bronchial lumen is also a matter of debate. Many authors agree that the average time should be from 6 to 12 months, provided that there are no complications requiring stent retrieval or re-stenting [18–19]. However, Fonesca et al. reported

1 to 7 years as follow-up periods for lung recipients with stents [20]. Often, removing a stent from the airway lumen, especially for metallic stents, is connected with certain technical difficulties associated with the risk of breaking the stent frame during retrieval, which ultimately leads to bleeding and bronchial wall rupture. The use of biodegradable materials in stent fabrication makes it possible to avoid the stent removal procedure.

One of the main materials used in the creation of biodegradable stents is polydioxanone (PDS). This material retains mechanical strength for 6 weeks and completely hydrolyzes after 3–4 months [21].

In 2011, Lischke et al. used PDS stents in 6 lung recipients with central bronchial stenosis [22]. The stents made from PDS had a range of standard sizes, were uncovered and had a braided shape. Median stent diameter ranged from 8 to 17 mm and median length from 12 to 30 mm. A total of 12 implantations were performed. In 4 patients, re-stenting had to be resorted to due to recurrent stenosis. Median time to any re-stenting was 5 months. On average, the stent was completely degraded 4 months after implantation.

DRUG-ELUTING STENTS

The use of drug-eluting stents may be a way to potentially reduce the incidence of stenting-associated complications, as well as increase the therapeutic effect of this intervention.

As mentioned above, the growth of granulation tissues along the proximal and distal edges of stents that are implanted in the lumen of the tracheobronchial tree is primarily associated with local inflammatory response, the final link of which is fibroblast proliferation. A similar mechanism is encountered in cardiovascular surgery when stents are implanted in the coronary lumen. Drug-eluting stents have found wide application in endovascular interventions. The use of drugs that reduce fibroblast proliferation ultimately reduces the number of complications associated with coronary artery stenting [23–26].

Zhu et al. experimentally evaluated the effect of mitomycin-eluting stents on the development of tracheal stenosis in rabbits [25]. The stent material was a mixture of polylactide (PLA) and polycaprolactone (PCL). Implantation was performed through a tracheotomy with stent suturing. In order to enhance tracheal tissue damage and, as a consequence, fibroblast proliferation, thermal exposure via monopolar coagulation was used. For mitomycin-eluting stents, the incidence of stenosis was lower than in the control groups (tracheal damage without stent implantation and use of a silicone stent without drug coating).

Chao et al. implanted cisplatin-eluting PCL tracheal stents in rabbits [26]. The stents were implanted through tracheotomy. Follow-up was 5 weeks. After the indicated time, no signs of stenosis were revealed at autopsy. Histological study showed the presence of ciliated epithelium with minimal leukocytic infiltration of the submucosal layer where the stent was located.

One of the main problems arising in the use of drug-eluting stents is uncontrolled and, often, instant release of the drug. Lee et al. described a technique for polydopamine-mediated immobilization of various molecules on the surface of a PLA stent [27].

Jumat et al. evaluated the degree of saturation and release of everolimus using polydopamine [28]. PLA polymer was used as a material. Everolimus immobilization on the surface of the material was evaluated using electron microscopy. Thus, when an intermediate layer of polydopamine was used, the amount of everolimus on the surface was significantly higher. The rate of everolimus release in buffer solution at 37 °C was lower in polydopamine-coated samples.

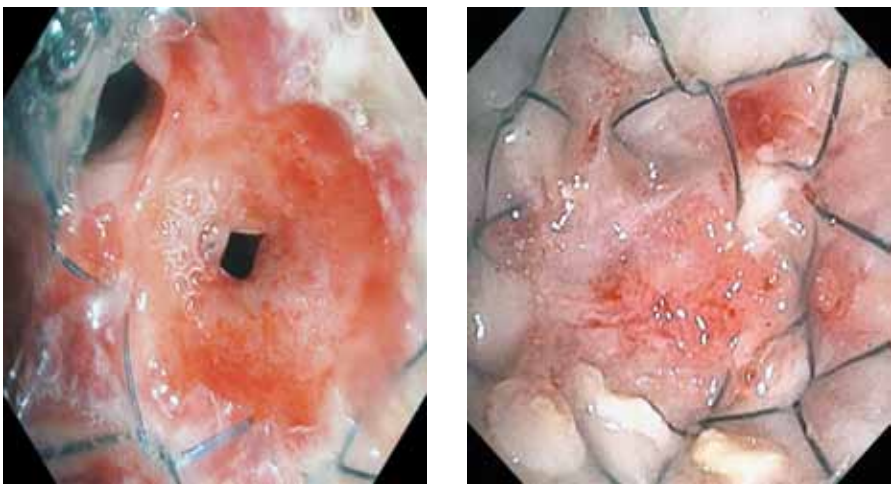

Fig. 4. Granulation tissue growth along the distal edge of the nitinol stent with stenosis formation

FABRICATION OF A 3D PRINTED DRUG-ELUTING STENT

Stents used in interventional pulmonology have standardized sizes and shapes. As a rule, each manufacturer has different stent options, differing in length and diameter. One of the main reasons causing stent migration is the mismatch between the anatomy of the tracheobronchial tree of a particular patient and the stent. In addition, stents with factory parameters do not take into account the localization of lobar and segmental bronchi, and the angle of departure.

Often, in order to adapt standard stents to the patient's anatomy, it is necessary to shorten the stent, create additional holes, and sew several prostheses [29]. These interventions lead to breach of design features, especially when using stents with weave.

Fig. 5. Stent model designed for subsequent printing

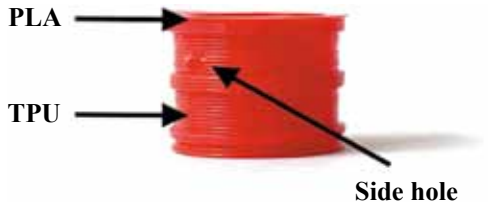

Fig. 6. Model of a 3D printed stent made by 3D printing (arrows indicate: PLA, polylactide rings; TPU, stent frame made of thermoplastic polyurethane; side hole, holes created on both sides of the stent in order to facilitate implantation)

Fig. 7. Left, stent before creation of polydopamine intermediate layer; right, stent with polydopamine intermediate layer

3D printing, also referred to as additive manufacturing, is a technology for manufacturing parts based on creating a physical object from an electronic model by adding material layer by layer. 3D printing of products has found wide application in medicine [30–31]. A model of the patient's tracheobronchial tree is created using CT scan data. Next, using modeling programs, a model of the stent is created with subsequent printing [32].

Fused deposition modeling (FDM) is a widely used additive manufacturing technique that creates three-dimensional objects through sequential, layer-by-layer application of plastic material. This technology has a number of advantages, including relatively low cost, a wide range of plastic materials, ability to print large objects, and availability of open source printers. It also allows for the creation of personalized models for each specific patient, which is critical to achieving the best possible treatment outcome.

Using a computer Aided Design (CAD) software solution, a 3D model of endotracheal stents was created, trying to combine the strengths of the most common stents in clinical practice (Fig. 5). The goal was to create a flexible stent similar to the Dumon stent, but with a strong framework capable of absorbing everolimus.

Thermoplastic polyurethane was chosen as the elastic base. The second plastic was polylactide, due to its high rigidity and hygroscopicity. After preparing the model for printing, a 3D printer that is capable of printing 2 different materials simultaneously was used.

Plastic filament was loaded into the printer and fed into the print head of the 3D printer. When exposed to the extruder, the plastic was extruded through the nozzle as a thin filament that was sequentially applied to the surface of the substrate. After each layer was applied, the plastic was cooled, causing it to solidify. This ensured the structural integrity of the layer before the next layer was applied. Gradually, layer by layer, the desired 3D object was formed (Fig. 6).

The fabrication of an everolimus-eluting stent was carried out with creation of an intermediate polydopamine layer. In order to polymerize dopamine, the stents were placed in the Tris-HCl buffer with pH 7.4. Also, dopamine hydrochloride was added to the solution at a ratio of 2 mg per 1 mL of buffer. Ammonium persulfate was used as a reaction catalyst and oxidant in a 1:2 ratio to dopamine hydrochloride [33]. Polymerization reaction was carried out at room temperature and in a dark room. After 24 hours, the color of the solution and stents changed to dark brown (Fig. 7).

The stents were washed in distilled water and placed in everolimus solution for 24 hours. Afterwards, the stents were removed and washed again in distilled water.

The microstructure of the samples was studied using field emission scanning electron microscopy (FE-SEM) on a Hitachi SU8000 electron microscope. Images were

captured in the mode of secondary electron registration at an accelerating voltage of 2 kV. Before imaging, the samples were placed on the surface of an aluminum table with a diameter of 25 mm, fixed with a conductive carbon tape and a conductive carbon layer with a 20 nm thickness was sprayed on it (Fig. 8).

CONCLUSION

Airway complications, particularly bronchial stenoses, reduce the quality of life and survival among lung recipients. The problem of airway complications is associated with the surgical aspects of transplantation, including the technique of procurement, organ preservation technique, bronchial anastomosis methods, the course of the postoperative period, including duration of ventilation, rejection episodes, occurrence of infections, immunosuppressive therapy regimen, and pathophysiological processes associated with bronchial wall ischemia.

It is worth noting that due to the high frequency of recurrences, there is no consensus on effective ways of correcting bronchial stenosis in lung recipients. One of the main lumen restoration methods is bronchial stenting.

The use of 3D printing makes it possible to create a stent that closely replicates the anatomy of the patient's airways. The materials used in 3D printing can be biodegradable.

Being an antiproliferative drug, everolimus can reduce fibroblast proliferation, thereby inhibiting granulation tissue growth. This property is actively used in endovascular surgery when creating drug-eluting coronary stents.

This article presents the experience of fabricating 3D-printed drug-eluting bronchial stents. Evaluation of the in vivo effect of these stents on experimental animals is the next stage of our research.

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The authors declare no conflict of interest.

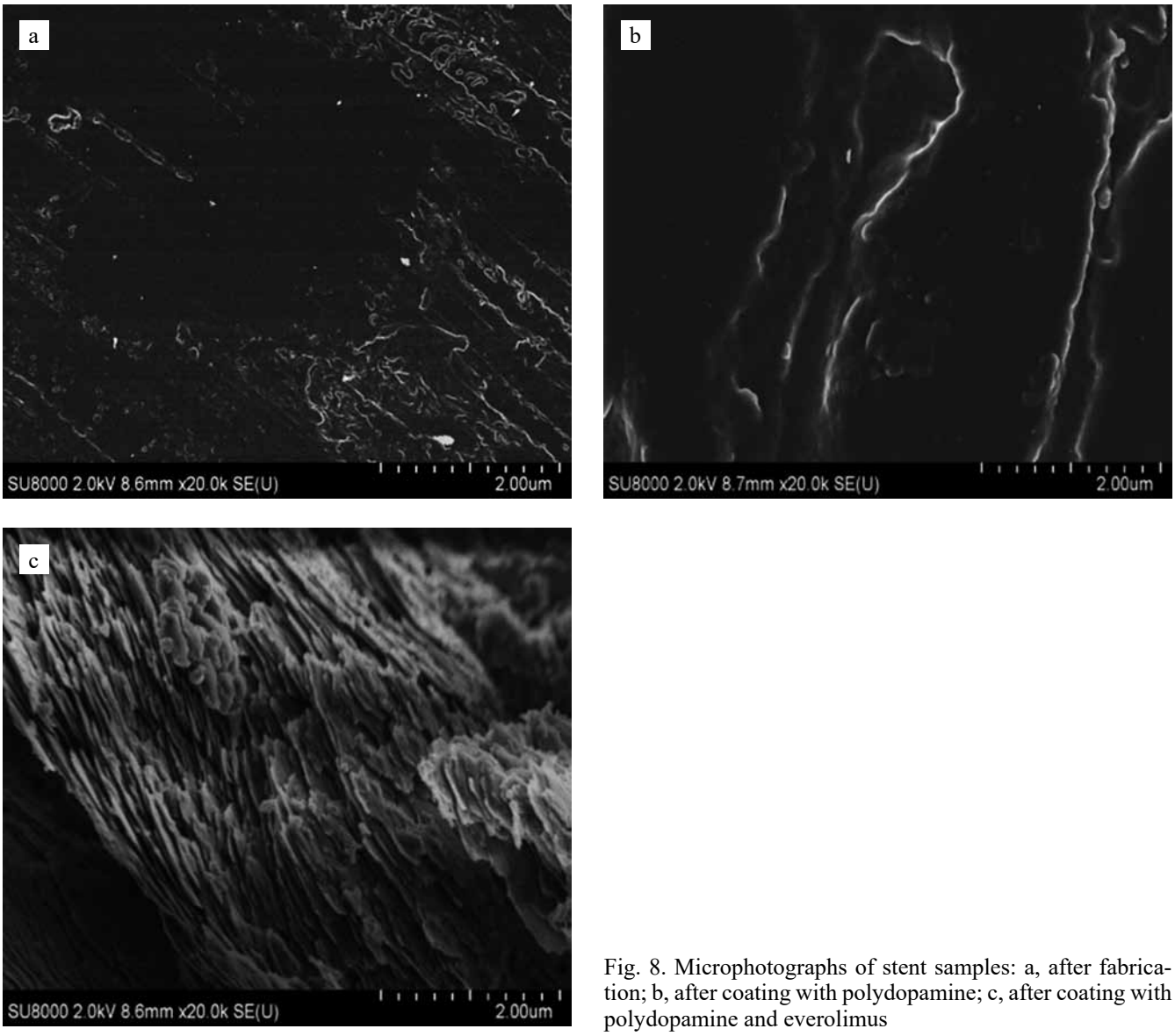

Fig. 8. Microphotographs of stent samples: a, after fabrication; b, after coating with polydopamine; c, after coating with polydopamine and everolimus

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STRATEGY FOR PROPHYLACTIC APPLICATION OF PERIPHERAL VA-ECMO IN TRANSPLANTATION INVOLVING EXPECTED EXTREMELY PROLONGED ISCHEMIA TIME

V.N. Poptsov, V.M. Zakharevich, E.A. Spirina, A.I. Skokova, A.K. Solodovnikova, A.S. Ignatkina, A.A. Kuznetsova, G.B. Glinkin

Shumakov National Medical Research Center of Transplantology and Artificial Organs, Moscow, Russian Federation

Heart transplantation (HT) with extremely prolonged (>6 hours) graft ischemia is associated with severe cardiac graft dysfunction. The high efficiency of prophylactic (preoperative initiation) veno-arterial extracorporeal membrane oxygenation (VA-ECMO) to prevent severe hemodynamic disorders during cardiac surgery has been demonstrated. **Objective:** to determine the effect of prophylactic VA-ECMO on the perioperative period in HT with an expected graft ischemia >6 hours. **Materials and methods.** Thirty-eight recipients (33 (86.8%) males and 5 (13.2%) females), age 11–66 (44.7 ± 12.0) years (median 48.0 years) were examined. Pre-transplant mechanical circulatory support (MCS) using peripheral VA-ECMO was applied in 15 (39.5%) recipients, in 6 of whom by prophylactic technique. The recipients (n = 38) were divided into 3 groups: 1) "no pre-HT VA-ECMO" (n = 23); 2) "pre-HT VA-ECMO" (n = 9) – pre-transplant VA-ECMO as a bridge to HT; 3) "prophylactic VA-ECMO" (n = 6). **Results.** In "prophylactic VA-ECMO" group, extracorporeal circulation (ECC) (94.0 [85.5; 102.8] min) and reperfusion time (20.0 [18.3; 27.6] min) were shorter ($p < 0.05$) compared to "no pre-HT VA-ECMO" (161.0 [122; 191.5] and 60.0 [55.3; 70.5] min) and "pre-HT VA-ECMO" (127.0 [117; 150.3] and 35.0 [27.8; 48.8] min) groups. The vasoactive-inotropic score was lower ($p < 0.05$) in "pre-HT VA-ECMO" and "prophylactic VA-ECMO" groups compared to recipients in "no pre-HT VA-ECMO" group, 12.1 [11.2; 14.0] and 12.5 [11.7; 14.8] *vs.* 16.0 [15.0; 18.5], respectively. The groups did not differ in terms of incidence of severe primary dysfunction. The "pre-HT VA-ECMO" and "prophylactic VA-ECMO" groups were characterized by shorter duration of mechanical ventilation (MV) compared with "no pre-HT VA-ECMO" group (11.7 [10.0; 16.5] and 12.7 [11.3; 18.4], respectively, *vs.* 14.5 [13.0; 19.3]). The "no pre-HT VA-ECMO" and "prophylactic VA-ECMO" groups did not differ in the need for postoperative MST, 21.7% and 16.7%, respectively. The groups did not differ in terms of length of stay in the intensive care unit (ICU) and in-hospital mortality – 0% ("prophylactic VA-ECMO") and 8.7% ("no pre-HT VA-ECMO") and 11.1% ("pre-HT VA-ECMO"), respectively. **Conclusion.** Prophylactic VA-ECMO in HT with extremely prolonged cardiac graft ischemia reduces ECC duration, reperfusion period, postoperative mechanical ventilation period, and the need for inotropic therapy.

Keywords: heart transplantation, prolonged ischemia time, prophylactic VA-ECMO.

INTRODUCTION

Suspected prolonged donor heart ischemic time is one of the criteria for expanded heart donation [1]. Although the limits of acceptable duration of donor heart ischemia have not yet been defined and continue to be the subject of scientific research, international guidelines state that the cardiac graft ischemia should not exceed 4 hours [2, 8]. Earlier studies have shown that ischemic time >4 hours significantly increases the risk of severe primary graft dysfunction requiring mechanical circulatory support (MCS) [3]. Some successful transplantations with cardiac graft ischemic time lasting for 4–6 hours and more demonstrates the possibility of effective transplantation with cold storage duration exceeding the recommended threshold (≤4 hours) [1, 4–7].

Prophylactic application of VA-ECMO during cardiac surgery is considered as one of the promising directions for improving surgical outcomes in patients with high surgical risk [9, 10].

The prerequisite for this study was the assumption that HT with an expected excessively long (>6 hours) cardiac graft ischemia under prophylactic VA-ECMO will contribute to the maintenance of systemic hemodynamics in the pre-perfusion period, reduce the reperfusion time (time interval between aortic clamp removal and end of ECC), total duration of ECC, reduce the dosage of cardiotonic drugs, and provide a timely transition from artificial to assisted circulation in case of severe early graft dysfunction.

The **objective** of the study was to determine the effect of prophylactic VA-ECMO on the course of the periope-

Corresponding author: Vitaly Poptsov. Address: 1, Shchukinskaya str., Moscow, 123182, Russian Federation. Phone: (495) 190-35-62. E-mail: poptsov_vit@mail.ru; livertranspl@mail.ru

rative period in HT with an expected duration of cardiac graft ischemic time >6 hours.

MATERIALS AND METHODS

The study included 38 recipients (33 (86.8%) males and 5 (13.2%) females) aged 11 to 66 (44.7 ± 12.0, median 48.0 years) who underwent primary (n = 37 (97.4%)) or repeat (n = 1 (2.6%)) HT (retransplantation) from January 1, 2011 to December 31, 2021) with cardiac graft ischemia lasting for more than 6 hours, which made up 2.5% of the total number of HT (n = 1500) during the analyzed period. In all observations, transplantation with extremely prolonged (≥6 hours) ischemia was due to the distance of the donor base from the transplant center.

The main heart pathology leading to chronic heart failure (CHF) and the need to perform HT were dilated cardiomyopathy (n = 20, 52.6%), coronary heart disease (CHD) (n = 16, 42.1%), restrictive cardiomyopathy (n = 1, 2.6%), and long-term irreversible cardiac graft dysfunction (n = 1, 2.6%)). CHF severity corresponded to stage IIA (n = 2, 5.3%), IIB (n = 25, 65.8%), and III (n = 11, 28.9%)) according to the Strazhesko–Vasilenko classification or to NYHA functional class 3 (n = 4, 10.5%)) and 4 (n = 34, 89.5%)) (3.8 ± 0.4). HT urgency corresponded to IA (n = 18, 47.4%), IB (n = 5, 13.2%)) or 2 (n = 15, 39.4%)) status according to the United Network for Organ Sharing (UNOS) algorithm.

Short-term pre-transplant MCS using peripheral VA-ECMO was applied in 15 (39.5%)) recipients, in 6 of them according to the **prophylactic technique**, in 4 (10.5%)) – long-term MCS by implantable left ventricular bypass method. VA-ECMO by pre-transplant MCS technique lasted for 1–6 (2.1 ± 0.8) days (n = 9), by prophylactic technique for 22–73 (44 ± 12) minutes (n = 6).

Patients were divided into 3 groups according to the absence or use of pre-transplant short-term MCS using peripheral VA-ECMO: (1) The "No pre-HT VA-ECMO" group (n = 23) consisted of those without pre-transplant VA-ECMO; (2) the "Pre-HT VA-ECMO" group (n = 9) comprised of those with pre-transplant VA-ECMO as a bridge to HT; (3) the "Prophylactic VA-ECMO" group (n = 6) included those with pre-transplant VA-ECMO using prophylactic VA-ECMO application technique.

For transplantation, we used hearts from brain-dead donors, whose condition was diagnosed in strict accordance with regulatory documents.

For VA-ECMO, we used the following perfusion devices for extracorporeal circulation: Medtronic BioConsole, RotaFlow Console, Cardiohelp-i, Medos. To fill the extracorporeal circuit, we used official balanced electrolyte solutions, up to 2000 mL with the addition of 5000 units of unfractionated heparin.

Peripheral femoral cannulation technique was used in all cases. Single-lumen, reinforced peripheral venous cannulas of 21–26 F size were used for blood drainage into the extracorporeal circuit depending on the recipient's anthropometric parameters. The venous cannula was installed at a depth of 30–35 cm from the skin surface and was determined by the recipient's growth parameters. The depth of this cannula location in the inferior vena cava was controlled using transesophageal echocardiogram to avoid competition with the venous cannula of the ECC circuit.

To return arterialized blood from the extracorporeal circuit to the systemic circulation, arterial peripheral femoral cannulas of 15–17 F size were used, depending on the recipient's anthropometric parameters, placed through the common femoral artery.

In prophylactic application of VA-ECMO, ECMO volumetric flow rate in the pre-perfusion period ranged from 1.2 to 1.5 L/min. In the postperfusion period, the flow rate depended on the initial function of the cardiac graft. With adequate graft functioning, ECMO volumetric flow rate was maintained at 1.0–1.5 L/min for no more than 3 days (protective mode). In cases of primary graft dysfunction, the volumetric flow rate and VA-ECMO duration depended on the nature and severity of its pumping dysfunction.

Study data was statistically processed using Microsoft Excel spreadsheets and application packages Statistica for Windows 7.0 (Start Soft Inc. USA), Biostat and SPSS. The obtained statistical data were combined into variation series according to the nature of distribution into research groups. The obtained data were presented in the form of quantitative (numerical) and categorical indicators. Normality of distributions was assessed using the Kolmogorov–Smirnov test. The values of numerical indicators are presented as mean with standard deviation (M ± σ), median (Me) with lower [Q1 (25%)] and upper [Q3 (75%)] quartiles. Categorical measures are presented as absolute values and percentages. Depending on the normality of distribution, the comparison of two groups by quantitative index was performed using Mann–Whitney U test or Student's t-test. A difference of $p < 0.05$ was considered significant. Pearson's chi-squared test and Fisher's exact test were used to compare categorical indicators.

RESULTS

A comparative analysis of the pre-transplant status of **recipients** in the 3 studied groups showed that with no differences in age, sex, anthropometric indices (weight, body surface area, BMI), nature of the underlying disease, and clinical manifestations of CHF were more ($p < 0.05$) pronounced in the recipients in whom VA-ECMO was used before HT ("Pre-HT VA-ECMO" and "Prophylactic VA-ECMO" groups) (Table 1). Pre-transplant impairments in systemic and central hemodynamics before MCS were more significant in "Pre-HT VA-ECMO" group, as expressed by significantly low mean blood pressure, PVR, CI and significantly high values of DPP, mPAP, PCWP compared to "No pre-HT VA-ECMO"

group or to both "No pre-HT VA-ECMO" and "Prophylactic VA-ECMO" groups. The pre-transplant laboratory results demonstrated significantly lower preoperative Hb, total protein, thrombocythemia and higher ($p < 0.05$) levels of urea, total bilirubin, AST, INR in "Pre-HT VA-ECMO" group compared to "No pre-HT VA-ECMO" group or both "No pre-HT VA-ECMO" and "Prophylactic VA-ECMO" groups, which reflected the severity of preoperative multi-organ dysfunction.

Donors for the recipients in "Prophylactic VA-ECMO" group were significantly older compared to those for "No pre-HT VA-ECMO" and "Pre-HT VA-ECMO" groups (Table 2). In addition, 83.3% of donors in "Prophylactic VA-ECMO" group were female and had significantly lower body weight and donor weight-recipient weight ratio. Donors in "No pre-HT VA-ECMO" group had a shorter ($p < 0.05$) duration of MV compared to "Pre-HT VA-ECMO" group. The groups did not differ significantly in the nature of causes of brain death, need and dosages of inotropic/vasopressor therapy, global echocardiographic parameters (except for interventricular septum (IVS) thickness), number of extended donor factors, marginalization score (assessment scales Eurotransplant Donor Heart Score, Donor Risk Index Model, RADIAL score).

Table 1

Pre-transplant clinical characteristics and laboratory and instrumental findings for transplantation with cardiac graft ischemia >6 hours in recipients with and without pre-transplant VA-ECMO (n = 38)

Indicator	Cardiac graft ischemia >6 hours (n = 38)			Statistical significance (p)		
	No pre-HT VA-ECMO	Pre-HT VA-ECMO	Prophylactic VA-ECMO	A	B	C
1	2	3	4	5	6	7
Number of observations	23	9	6			
Age (years) M ± σ Me [Q1; Q3]	 49.0 ± 9.9 48.0 [46.0; 57.0]	 48.8 ± 11.9 52.0 [39.5; 57.0]	 50.5 ± 8.7 49.0 [45.0; 54.0]	0.962	0.738	0.770
Gender Female (n/%)	 1/4.3	 2/22.2	 2/33.3	0.184	0.100	1.000
Weight (kg) M ± σ Me [Q1; Q3]	 81.4 ± 15.6 79.5 [70.0; 88.3]	 84.8 ± 18.6 91.0 [74.8; 97.5]	 84.8 ± 20.5 88.5 [74.5; 98.8]	0.603	0.659	1.000
Body surface area (m^2) M ± σ Me [Q1; Q3]	 1.92 ± 0.23 1.90 [1.79; 2.10]	 2.01 ± 0.26 2.1 [1.90; 2.10]	 1.95 ± 0.28 2.0 [1.89; 2.10]	0.345	0.787	0.678
BMI (kg/m^2) M ± σ Me [Q1; Q3]	 26.7 ± 4.4 26.4 [24.2; 28.4]	 28.5 ± 6.7 28.5 [23.9; 31.8]	 28.8 ± 7.4 28.9 [23.1; 34.7]	0.378	0.376	0.936
Underlying disease: DCM (n/%) CHD (n/%)	 10/43.5 11/47.8	 4/44.4 5/55.6	 2/33.3 4/66.7	 1.000 1.000	 1.000 0.651	 1.000 1.000
NYHA FC M ± σ Me [Q1; Q3]	 3.1 ± 0.3 3.0 [3.0; 3.0]	 3.8 ± 0.5 4.0 [3.8; 4.0]	 3.9 ± 0.2 4.0 [4.0; 4.0]	**0.001**	**0.001**	0.652
RAP (mmHg) M ± σ Me [Q1; Q3]	 7.8 ± 3.8 7.0 [5.0; 10.0]	 12.6 ± 5.1 13.5 [8.8; 18.0]	 6.5 ± 2.3 6.0 [4.8; 7.8]	**0.007**	0.434	**0.017**
mPAP (mmHg) M ± σ Me [Q1; Q3]	 24.7 ± 8.1 24.0 [19.0; 27.0]	 31.2 ± 7.7 31.0 [26.8; 36.0]	 26.0 ± 4.8 26.5 [25.3; 27.5]	**0.047**	0.712	0.167
PCWP (mmHg) M ± σ Me [Q1; Q3]	 17.5 ± 4.9 15.0 [12.0; 18.0]	 22.0 ± 5.1 22.0 [18.0; 25.0]	 16.0 ± 2.8 15.0 [14.0; 17.0]	**0.028**	0.481	**0.022**

End of table. 1

1	2	3	4	5	6	7
CI (L/min/m^2) M ± σ Me [Q1; Q3]	1.95 ± 0.39 2.0 [1.7; 2.3]	1.61 ± 0.34 1.6 [1.2; 1.7]	1.88 ± 0.50 2.0 [1.8; 2.1]	**0.029**	0.714	0.232
TPG (mmHg) M ± σ Me [Q1; Q3]	7.2 ± 2.8 7.0 [5.0; 8.6]	8.5 ± 2.5 8.0 [7.5; 9.3]	8.5 ± 3.3 7.5 [6.0; 10.0]	0.234	0.037	1.000
PVR (Wood units) M ± σ Me [Q1; Q3]	2.1 ± 0.8 1.6 [1.3; 3.1]	2.8 ± 0.9 2.6 [2.1; 3.7]	2.5 ± 0.4 2.5 [2.3; 2.7]	**0.040**	0.250	0.460
LVEF (%) M ± σ Me [Q1; Q3]	25.7 ± 8.1 24.0 [18.0; 29.0]	20.1 ± 8.9 18.0 [13.0; 22.5]	17.5 ± 7.0 18.8 [12.3; 23.5]	0.097	0.032	0.559
Mitral regurgitation (grade) M ± σ Me [Q1; Q3]	1.9 ± 0.7 2.0 [1.7; 2.3]	2.8 ± 0.3 2.7 [2.4; 2.9]	2.3 ± 0.5 2.3 [2.2; 2.6]	**0.001**	0.202	**0.030**
Tricuspid regurgitation (grade) M ± σ Me [Q1; Q3]	1.8 ± 0.5 2.0 [1.0; 2.0]	2.7 ± 0.5 2.6 [2.3; 3.0]	2.2 ± 0.3 2.2 [2.1; 2.7]	**0.001**	**0.074**	**0.048**
Hb (g/dL) M ± σ Me [Q1; Q3]	13.4 ± 3.4 13.7 [12.1; 15.9]	11.0 ± 1.8 11.0 [9.8; 11.8]	14.9 ± 4.1 14.8 [14.5; 15.3]	**0.055**	0.487	**0.024**
Platelets (×10^9/L) M ± σ Me [Q1; Q3]	202.2 ± 73.5 194.0 [144.0; 240.0]	77.6 ± 36.4 74.0 [60.2; 85.4]	221.8 ± 39.7 232.0 [204.5; 249.3]	**0.001**	**0**.538	**0.001**
Total protein (mmol/L) M ± σ Me [Q1; Q3]	74.7 ± 6.0 76.3 [70.9; 78.5]	68.2 ± 5.5 66.0 [65.1; 72.0]	73.3 ± 2.5 73.1 [71.6; 74.7]	**0.009**	0.585	**0.055**
Urea (mmol/L) M ± σ Me [Q1; Q3]	8.2 ± 2.5 7.5 [6.3; 9.2]	10.5 ± 2.6 10.0 [8.2; 13.0]	8.1 ± 1.3 7.9 [7.2; 8.7]	**0.028**	0.926	**0.058**
Creatinine (μmol/L) M ± σ Me [Q1; Q3]	99.5 ± 29.9 91.9 [80.2; 121.8]	106.8 ± 25.2 103.5 [84.3; 125.4]	84.9 ± 23.7 85.7 [75.7; 94.9]	0.523	0.279	0.115
Total bilirubin (μmol/L) M ± σ Me [Q1; Q3]	26.9 ± 20.2 20.7 [13.7; 35.0]	46.5 ± 18.9 41.2 [20.8; 62.0]	23.1 ± 7.9 20.8 [17.1; 26.7]	**0.018**	0.659	**0.014**
INR M ± σ Me [Q1; Q3]	1.19 ± 0.11 1.20 [1.10; 1.30]	1.46 ± 0.48 1.40 [1.20; 1.64]	1.25 ± 0.23 1.30 [1.10; 1.40]	**0.015**	0.359	0.340

Note. A, p-value of "No pre-HT VA-ECMO" and "Pre-HT VA-ECMO"; B, p-value of "No pre-HT VA-ECMO" and "Prophylactic VA-ECMO"; C, p-value of "Pre-HT VA-ECMO" and "Prophylactic VA-ECMO"; BMI, body mass index; CHF, chronic heart failure; DCM, dilated cardiomyopathy; CHD, coronary heart disease; FC, functional class; RAP, right atrial pressure; mPAP, mean pulmonary artery pressure; PCWP, pulmonary artery wedge pressure; CI, cardiac index; TPG, transpulmonary pressure gradient; PVR, pulmonary vascular resistance; LVEF, left ventricular ejection fraction; Hb, hemoglobin; INR, international normalized ratio.

Analysis of the course of the **perioperative period** showed that in "Prophylactic VA-ECMO" recipients, the duration of ECC and reperfusion period ("aortic clamp removal-to-end of ECC" interval) were shorter ($p < 0.05$) compared to "No pre-HT VA-ECMO" and "Pre-HT VA-ECMO" groups (Table 3). Recipients in "Pre-HT VA-ECMO" and "Prophylactic VA-ECMO" groups had lower doses of cardiotonic/vasopressor therapy medications ($p < 0.05$) compared to those in "No pre-HT VA-ECMO" group. Recipients in the study groups did not differ in the incidence of severe primary graft dysfunction requiring post-transplant VA-ECMO. Due to the development of severe primary dysfunction, 4 recipients in "No pre-HT VA-ECMO" group needed to be connected to the peripheral VA-ECMO system in the early postperfusion period. In all, "Pre-HT VA-ECMO" recipients ($n = 9$) and in 5 of 6 "Prophylactic VA-ECMO" recipients, MCS was continued in the posttransplant period in a safety mode (blood flow rate <1.2–1.5 L/min). ECMO volumetric flow rate and duration of posttransplant VA-ECMO were significantly lower in "Prophylactic VA-ECMO" group. Recipients from "Prophylactic VA-ECMO" group and from "No pre-HT VA-ECMO" group did not differ in terms of volume of intra- and postoperative blood loss and the need for transfusion therapy. Accordingly, the values of these parameters were higher ($p < 0.05$) in "Pre-HT VA-ECMO" group compared to "Prophylactic VA-ECMO" and "No pre-HT VA-ECMO" groups. The duration of postoperative MV was shorter ($p < 0.05$) in "Pre-HT VA-ECMO" and "Prophylactic VA-ECMO" groups. Renal replacement therapy (RRT) was used more frequently ($p < 0.05$) in "Pre-HT VA-ECMO" group, 66.7%. Recipients from "No pre-HT VA-ECMO" and "Prophylactic VA-ECMO" groups did not differ in the need for postoperative RRT, with the frequency of use being 21.7% and 16.7%, respectively. The groups were not statistically different in terms of duration of postoperative ICU treatment and in-hospital mortality. There was no mortality in "Prophylactic VA-ECMO" group.

DISCUSSION

HT with prolonged graft ischemia is characterized by longer reperfusion period (≥1 hour) and, accordingly, ECC duration, which is associated with gradual, slow restoration of myocardial contractility and pumping function of the heart transplant. Prolonged ECC is an important factor in the development of multi-organ dysfunction and the cause of eventful postoperative period in heart recipients [11]. In addition, HT with expected prolonged cardiac graft ischemia is associated with increased risk of impaired pumping function of the cardiac graft at the early stages of its functioning up to development of severe primary dysfunction due to severe manifestations of ischemia-reperfusion injury (IRI) [12]. Gradual, delayed recovery of myocardial contractility of the transplanted heart makes it necessary to use sympathomimetic drugs in high doses, which negatively affects early and long-term recipient survival [13]. In the absence of restoration of adequate pumping function of the cardiac graft, transition from ECC to different variants of assisted circulation is indicated. Excessive prolongation of ECC in an attempt to wait for rapid resolution of transplanted heart dysfunction and, accordingly, delay in timely withdrawal of ECC and initiation of assisted circulation increases the risk of unfavorable outcome after HT [3, 14]. Peripheral VA-ECMO is currently considered as the leading method of MCS in recipients with severe primary cardiac graft dysfunction [15].

Our previous experience with VA-ECMO as a short-term method of pre-transplant MCS has shown its versatility and efficacy both before and after HT in cases of severe primary graft dysfunction [16, 17].

Table 2

Results of anthropometric, anamnestic, laboratory, and echocardiographic findings of the heart donor for transplantation with cardiac graft ischemia >6 hours in recipients with and without pre-transplant VA-ECMO (n = 38)

Indicator	Cardiac graft ischemia >6 hours (n = 38)			Statistical significance (p)		
	No pre-HT VA-ECMO	Pre-HT VA-ECMO	Prophylactic VA-ECMO	A	B	C
1	2	3	4	5	6	7
Number of observations	23	9	6			
Age (years) M ± σ Me [Q1; Q3]	 42.6 ± 7.5 44.0 [37.8; 51.8]	 41.0 ± 6.2 43.5 [35.5; 46.3]	 49.0 ± 6.3 48.0 [44.5; 52.5]	0.575	0.066	0.030
Gender Female (n/%)	 0.0/0.0	 0/0.0	 5/83.3	–	0.001	0.002
Weight (kg) M ± σ Me [Q1; Q3]	 81.5 ± 12.0 80.0 [71.3; 89.3]	 77.9 ± 10.8 77.5 [70.0; 86.3]	 66.5 ± 15.8 60.0 [59.0; 67.5]	0.440	0.016	0.085

End of table. 2

1	2	3	4	5	6	7
Donor-to-recipient weight ratio M ± σ Me [Q1; Q3]	1.00 ± 0.16 1.00 [0.92; 1.10]	0.92 ± 0.26 1.0 [0.70; 1.06]	0.80 ± 0.19 0.8 [0.70; 0.93]	0.297	0.014	0.351
Causes of brain death: Stroke (n/%)	20/87.0	8/77.8	6/100.0	1.000	1.000	1.000
ICU/MV (days) M ± σ Me [Q1; Q3]	1.9 ± 0.9 2.0 [1.0; 2.0]	3.7 ± 2.7 3.0 [2.0; 4.5]	2.3 ± 0.9 2.5 [1.8; 3.0]	0.007	0.343	0.351
VIS (points, max) M ± σ Me [Q1; Q3]	35.4 ± 27.6 29.8 [20.0; 59.8]	29.9 ± 32.3 24.0 [11.4; 29.0]	36.7 ± 16.3 35.0 [22.5; 47.5]	0.632	0.914	0.644
IVS (cm) M ± σ Me [Q1; Q3]	1.20 ± 0.24 1.20 [1.00; 1.30]	1.29 ± 0.21 1.2 [1.0; 1.5]	1.10 ± 0.08 1.1 [1.0; 1.1]	0.332	0.329	0.056
LVEDV (mL) M ± σ Me [Q1; Q3]	103.0 ± 27.6 100.0 [87.3; 121.0]	95.6 ± 18.3 91.0 [82.5; 101.5]	94.8 ± 15.4 92.5 [85.8; 101.5]	0.465	0.494	0.931
LVEF (%) M ± σ Me [Q1; Q3]	63.2 ± 6.3 64.0 [59.0; 67.0]	61.5 ± 3.7 61.0 [59.8; 64.3]	64.0 ± 1.2 64.0 [63.0; 65.0]	0.456	0.7862	0.137
Blood Na^+ (mmol/L) M ± σ Me [Q1; Q3]	144.8 ± 8.6 140.0 [139.0; 150.0]	145.8 ± 11.9 144.0 [138.5; 150.0]	144.5 ± 4.9 145.5 [142.8; 146.3]	0.793	0.915	0.806
Hb (g/dL) M ± σ Me [Q1; Q3]	12.4 ± 2.8 12.3 [10.5; 14.0]	11.9 ± 2.4 11.8 [9.7; 13.6]	9.7 ± 1.9 9.7 [9.1; 10.3]	0.641	0.035	0.083
Total protein (g/L) M ± σ Me [Q1; Q3]	61.5 ± 13.4 66.5 [55.0; 67.8]	63.8 ± 9.6 66.0 [56.6; 70.0]	60.5 ± 13.8 65.5 [60.8; 70.3]	0.643	0.702	0.592
Expanded heart donation factor (n), M ± σ Me [Q1; Q3]	2.1 ± 0.3 2.0 [1.8; 2.1]	2.1 ± 0.4 2.0 [1.9; 2.1]	2.2 ± 0.5 2.1 [1.8; 2.3]	1.000	0.534	0.674
Eurotransplant Donor Heart Score (points) M ± σ Me [Q1; Q3]	18.6 ± 5.6 18.0 [16.3; 20.5]	19.7 ± 6.2 19.1 [17.3; 22.0]	22.8 ± 7.4 20.6 [18.2; 23.5]	0.631	0.429	0.395
Donor Risk Index Model (points) M ± σ Me [Q1; Q3]	6.4 ± 1.9 6.0 [5.0; 7.2]	6.9 ± 2.5 6.5 [5.2; 8.0]	7.4 ± 2.7 7.2 [5.8; 8.5]	0.545	0.302	0.719
RADIAL score (points) M ± σ Me [Q1; Q3]	2.7 ± 0.7 2.6 [2.4; 3.0]	2.8 ± 0.9 2.6 [2.5; 3.2]	3.1 ± 0.7 3.0 [2.6; 3.4]	0.740	0.223	0.504

Note. A, p-value of "No pre-HT VA-ECMO" and "Pre-HT VA-ECMO"; B, p-value of "No pre-HT VA-ECMO" and "Prophylactic VA-ECMO"; C, p-value of "Pre-HT VA-ECMO" and "Prophylactic VA-ECMO"; TBI, traumatic brain injury; CPR, cardiopulmonary resuscitation; ICU, intensive care unit; MV, mechanical ventilation; min, minimum; max, maximum; VIS, vasoactive inotropic score; IVS, interventricular septum; LVEDV, left ventricular end-diastolic volume; LVEF, left ventricular ejection fraction; Hb, hemoglobin.

In this regard, it was assumed that HT, under prophylactic MCS using peripheral VA-ECMO, will provide hemodynamic stability not only in the pre-perfusion period, but also in the early posttransplant period when restoring the pumping function of the transplanted heart. A scheduled transition from ECC to posttransplant MCS by peripheral VA-ECMO can reduce reperfusion time and ECC duration, use of sympathomimetic cardiotonics in lower doses and maintain adequate level of systemic circulation in cases of gross impairment of pumping function of the heart transplant due to severe primary dysfunction.

The study demonstrated that patients in whom VA-ECMO was used in the pre-transplant period, regardless of its technique (therapeutic (bridge to HT) or prophylactic) had more severe manifestations of CHF, impaired central and systemic hemodynamics, which justified the use of preoperative MCS. Accordingly, the most severe pre-transplant hemodynamic impairments were in patients in whom VA-ECMO was used as a mechanical short-term bridge to HT [18]. Transplant centers with a high volume of HT have clinical and organizational opportunities to use VA-ECMO as a method of short-term MCS before HT with guaranteed survival to heart transplantation in optimal time (up to 10–14 days) [17]. However, it should be taken into account that patients with pre-transplant VA-ECMO belong to the most severe category of heart recipients with high risk of perioperative complications and early post-transplant survival rates lower than in recipients without preoperative MCS [19, 20].

One of the developing directions of perioperative MCS in cardiac surgery is the strategy of prophylactic application of VA-ECMO in patients at high risk of intraoperative life-threatening hemodynamic impairments of various genesis or development of postcardiotomy acute heart failure (AHF) [21–23]. International guidelines on prophylaxis and treatment of postcardiotomy AHF consider the prophylactic application of VA-ECMO as one of the highly effective measures for early correction of hemodynamic impairments caused by this critical complication [24].

We assumed that prophylactic connection of the patient to a VA-ECMO circuit immediately before HT surgery would ensure guaranteed maintenance of systemic hemodynamics both in the pre-perfusion and early post-transplant periods. The increased risk of hemodynamic destabilization in patients with severe manifestations of CHF due to progression of myocardial failure and/or life-threatening cardiac arrhythmias at the most critical stages of surgical intervention before ECC – sternotomy, isolation and placement of purse-string sutures on the vena cava, vena cava cannulation – was taken into account. There is increased risk of such an unfavorable scenario in patients in whom HT is a repeated surgical intervention and prolonged and traumatic cardiolysis is required due to severe adhesions in the pericardial cavity. Since HT with expected prolonged ischemia may be accompanied by severe IRI and primary graft dysfunction, prophylactic use of VA-ECMO guarantees the maintenance of systemic hemodynamics in the event of this complication.

In addition, preoperative initiation of VA-ECMO provides rapid timely transition to assisted circulation, reducing reperfusion and ECC time, as well as the intensity of sympathomimetic cardiotonic therapy, reducing the risk of severe multi-organ failure [25].

The study demonstrated that when VA-ECMO was started preemptively immediately before HT, the transplant cardiac surgery itself proceeded with significantly less blood loss and transfusion therapy compared to recipients in whom VA-ECMO was used as a pre-transplant MCS (bridge to transplant). In addition, recipients with and without preoperative VA-ECMO did not differ in these parameters. It was also noted that recipients with postoperative VA-ECMO had a shorter duration of postoperative MV, which is due to the possibility of safe transfer to spontaneous breathing under extracorporeal circulation and gas exchange [26]. Thus, the study demonstrated the possibility of effective application of VA-ECMO as a prophylactic measure aimed at preventing intra- and postoperative life-threatening hemodynamic impairments when performing HT with excessively long (>6 hours) cardiac graft ischemia.

Table 3

Perioperative period for graft transplantation with cardiac graft ischemia >6 hours in recipients with and without pre-transplant VA-ECMO (n = 38)

Indicator	Cardiac graft ischemia >6 hours (n = 38)			Statistical significance (p)		
	No pre-HT VA-ECMO	Pre-HT VA-ECMO	Prophylactic VA-ECMO	A	B	C
1	2	3	4	5	6	7
Number of observations	23	9	6			
Graft ischemia (min) M ± σ Me [Q1; Q3]	 424.4 ± 48.9 414.0 [390.0; 449.5]	 413.4 ± 57.9 395.0 [364.0; 428.0]]	 426.5 ± 46.1 419.5 [405.8; 440.3]	0.591	0.925	0.651

End of table. 3

1	2	3	4	5	6	7
ECC (min) M ± σ Me [Q1; Q3]	173.3 ± 38.9 161.0 [122.0; 191.5]	121.3 ± 30.5 127.0 [117.0; 150.3]	94.3 ± 12.4 94.0 [85.5; 102.8]	**0.001**	**0.001**	**0.062**
"Aortic clamp removal/end of ECC" interval M ± σ Me [Q1; Q3]	66.3 ± 14.7 60.0 [55.3; 70.5]	35.3 ± 11.9 35.0 [27.8; 48.8]	22.3 ± 7.9 20 [18.3; 27.6]	**0.001**	**0.001**	**0.036**
Dopamine (max, μg/kg/min) M ± σ Me [Q1; Q3]	9.6 ± 2.9 8.5 [7.5; 10.3]	7.1 ± 2.4 7.0 [5.5; 9.0]	6.9 ± 1.0 8.0 [7.5; 8.0]	**0.029**	**0.035**	0.851
Adrenaline (max, μg/kg/min) M ± σ Me [Q1; Q3]	73.8 ± 25.9 65.0 [50.0; 80.0]	47.6 ± 17.8 40.0 [35.0; 55.0]	46.5 ± 15.2 42.5 [38.3; 60.0]	**0.009**	**0.021**	0.903
VIS (max) M ± σ Me [Q1; Q3]	16.5 ± 4.1 16.0 [15.0; 18.5]	12.3 ± 3.6 12.1 [11.2; 14.0]	12.0 ± 4.3 12.5 [11.7; 14.8]	**0.042**	**0.025**	0.886
Severe primary dysfunction (n/%)	4/17.4	0/0.00	1/16.7	0.303	1.000	0.400
MV (hours) M ± σ Me [Q1; Q3]	17.9 ± 7.1 14.5 [13.0; 19.3]	12.3 ± 5.4 11.7 [10.0; 16.5]	11.3 ± 5.8 12.7 [11.3; 18.4	**0.042**	**0.046**	0.736
Post-HT VA-ECMO (n/%)	4/14.5	9/100.0	6/100.0			
Post-HT VA-ECMO, (L/min) M ± σ Me [Q1; Q3]	3.3 ± 0.4 3.1 [3.3; 3.5]	2.3 ± 0.2 2.2 [2.0; 2.4]	1.8 ± 0.4 2.1 [1.6; 2.0]	**0.001**	**0.001**	**0.007**
VA-ECMO (hours) M ± σ Me [Q1; Q3]	116.6 ± 23.5 110 [105; 130.0]	63.6 ± 13.5 55.0 [50.0; 65.7]	47.4 ± 8.9 42.7 [38.7; 52.1]	**0.001**	**0.001**	**0.023**
VA-ECMO (>3 days) n/%	4/14.5	0/0.00	1/16.7	0.303	1.000	0.400
Blood loss (mL) M ± σ Me [Q1; Q3]	1081.3 ± 324.5 1010 [860.0; 1350.0]	3671.4 ± 849.8 3200 [2750.0; 5200.0]	835.0 ± 448.0 555.0 [465.0; 825.0]	**0.001**	0.137	**0.001**
Erythromass (mL) M ± σ Me [Q1; Q3]	570.4 ± 181.3 500.0 [350.0; 825.0]	1847.3 ± 643.2 1800.0 [1016.0; 3160.0]	610.5 ± 98.3 380.4 [320.3; 550.5]	**0.001**	0.609	**0.001**
FFP (mL) M ± σ Me [Q1; Q3]	1020.4 ± 427.1 950.0 [700.0; 1300.0]	3040.8 ± 744.3 2830.0 [2450.0; 4270.0]	960.7 ± 340.5 880.3 [800.5; 1150.4]	**0.001**	0.756	**0.001**
RRT (%)	5/21.7	6/66.7	1/16.7	**0.035**	1.000	0.119
ICU (days) M ± σ Me [Q1; Q3]	5.3 ± 3.2 5.0 [4.2; 6.0]	6.5 ± 3.8 6.0 [5.5; 7.8]	5.0 ± 2.9 4.7 [4.1; 6.2]	0.372	0.837	0.426
In-hospital mortality (n/%)	2/8.7	1/11.1	0/0.00	1.000	1.000	1.000

Note. A, p-value of "No pre-HT VA-ECMO" and "Pre-HT VA-ECMO"; B, p-value of "No pre-HT VA-ECMO" and "Prophylactic VA-ECMO"; C, p-value of "Pre-HT VA-ECMO" and "Prophylactic VA-ECMO"; ECC, extracorporeal circulation; VIS, vasoactive inotropic score; MV, mechanical ventilation; max, maximum; FFP, fresh frozen plasma; RRT, renal replacement therapy; ICU, intensive care unit.

CONCLUSION

Prophylactic VA-ECMO in HT with extremely prolonged ischemic time reduces ECC duration, reperfusion period, and postoperative MV period, and decreases the need for inotropic therapy.

The authors declare no conflict of interest.

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IMPLANTATION OF HEARTMATE III VENTRICULAR ASSIST DEVICES IN CHILDREN AFTER SURGICAL TREATMENT OF COMPLEX CONGENITAL HEART DEFECT: FIRST EXPERIENCE

K.V. Shatalov, M.V. Makhalin, M.A. Chupina, E.Z. Goluhova
Bakulev Center for Cardiovascular Surgery, Moscow, Russian Federation

Background. There is quite a high number of patients with advanced heart failure (HF) who have undergone surgical treatment for complex congenital heart defects. Implantation of mechanical circulatory support systems is the only treatment option for patients with refractory end-stage heart failure. Only a few centers have experience in implantation of ventricular assist devices (VAD) in children, which is a major challenge for modern pediatric cardiac surgery. **Objective:** to present our first experience of implantation of HeartMate III VADs in patients after surgical correction of complex congenital heart defects. **Materials and methods.** From 2021 to 2022, at Bakulev Center for Cardiovascular Surgery, four HeartMate III systems were implanted in children with advanced HF, who had previously undergone surgery for a complex congenital heart defect. In one case, aortic valve implantation was carried out simultaneously with VAD implantation. **Results.** All patients were discharged from the center. One patient developed right-sided heart failure intraoperatively, which required the use of a right ventricular assist device (RVAD) for 8 days. There were no complications from the central nervous system, bleeding, pump thrombosis, or infection. **Conclusion.** HeartMate III can be implanted in patients with body weight ≥21 kg and BSA ≥0.88 m^2. Children's tolerance to physical activity increases, they are fully adapted socially, and can attend school.

Keywords: advanced heart failure, ventricular assist device, congenital heart defect.

INTRODUCTION

Implantation of mechanical circulatory support (MSC) systems is often the only treatment option for patients with refractory end-stage heart failure (ESHF). Heart transplantation (HT) options are limited by organ shortages that cannot be overcome. Globally, the number of implantations of such systems is increasing every year. Successful use of VADs in the treatment of ESHF in adults has led to industrial innovations in VADs with a focus on reducing their size and the incidence of potential complications. This has set the stage for the use of new generation devices in pediatric practice. The use of VADs in children has increased significantly over the past decade, and newer generation continuous-flow devices are now replacing the older pulsatile-flow devices that were previously the only option for young patients. Despite the increase in pediatric VAD implants, the number of patients remains relatively small. There have been reports on cases of implantation of HeartMate 3 VADs in children with dilated cardiomyopathy (DCM) and after hemodynamic correction of complex congenital heart disease (CHD) that ended with the Fontan procedure. In review articles, there are isolated cases of HeartMate 3 implantation in CHD children, but there is no data on which defects and whether they were corrected before implantation [1].

MATERIALS AND METHODS

In 2021–2022, four HeartMate 3 systems were implanted in children who had previously undergone multiple surgeries for complex CHD (single right ventricle, double outlet right ventricle (DORV), transposition of the great arteries, and aortic stenosis) and who developed critical heart failure in the long term after surgery. Given the severity of the children's condition, shortage of donors and peculiarities of the legislative framework in the Russian Federation, VAD implantation was used as an alternative to heart transplantation.

Demographics, previous surgeries, and INTERMACS level are presented in Table 1.

Preoperative examination

All patients were examined according to a single protocol, which included the following investigations: ECG, chest X-ray, echocardiography, angiocardiography and coronary angiography with measurement of central hemodynamics, contrast-enhanced chest CT scan, biochemical and hematological blood tests.

ECG revealed that three patients had sinus rhythm with a heart rate corresponding to the age norm. In one patient with an implantable triple-chamber cardiac resynchronization therapy pacemaker (CRT-P), no pacemaker dysfunction was detected.

Corresponding author: Maksim Makhalin. Address: 135, Rublevskoe Shosse, 121552, Moscow, Russian Federation. Phone: (926) 220-31-10. E-mail: mmaxalin@mail.ru

Chest X-ray examination showed that all patients had increased heart size, cardiothoracic ratio ranged from 52% to 75%.

Echocardiographic study was performed according to the protocol adopted in our center, Bakulev Center for Cardiovascular Surgery. All patients showed a sharp decrease in contractility of the left or systemic ventricle and a significant increase in its linear dimensions. Two patients had no significant stenosis or valvular insufficiency, one patient had satisfactory mitral and tricuspid valve functions. One patient had a high peak aortic graft gradient. Echocardiographic data are presented in Table 2.

During angiocardiographic study, coronary anatomy and heart anatomy were assessed, and pressure in the heart chambers was measured (Fig. 1). Central hemodynamic parameters were measured using a Swan–Ganz catheter; the corresponding data are presented in Table 3.

Table 1

Preoperative patient characteristics

Patient characteristics:	Patient 1	Patient 2	Patient 3	Patient 4
Age	11 years, 5 months	9 years, 4 months	13 years, 7 months	12 years, 10 months
Gender	male	female	female	male
Body weight (kg)	35	21	49	53
BSA (m^2)	1.23	0.88	1.52	1.5
Diagnosis:	Single right ventricle	Double outlet right ventricle (DORV)	Transposition of the great arteries	Aortic stenosis
Surgeries undergone:	1. Transluminal balloon atrial septostomy 2. BCPA, dilatation of IAC, ligation of main PA. 3. Embolization of MAPCA. 4. Fontan procedure	1. Definitive surgical repair of DORV. 2. MV implantation, subaortic membrane resection. 3. MV second implantation, TV implantation. 4. Implantation of a dual-chamber pacemaker. 5. Implantation of a triple-chamber pacemaker – CRT-P	1. Transluminal balloon atrial septostomy. 2. SPAS, constriction of the main PA, ligation of PDA. 3. Arterial switch surgery	1. Aortic valve plasty. 2. Aortic valve implantation, LCA trunk and AIA stenting. 3. LCA stenting
INTERMACS level	4	4	4	4

Note. BSA, body surface area; BCPA, bidirectional cavopulmonary anastomosis; IAC, interatrial communication; PA, pulmonary artery; MAPCA, major aortopulmonary collateral artery; MV, mitral valve; TV, tricuspid valve; CRT-P, cardiac resynchronization therapy pacemaker – a biventricular pacemaker; SPAS, systemic-to-pulmonary artery shunt; PDA, patent ductus arteriosus; LCA, left coronary artery; AIA, anterior interventricular artery.

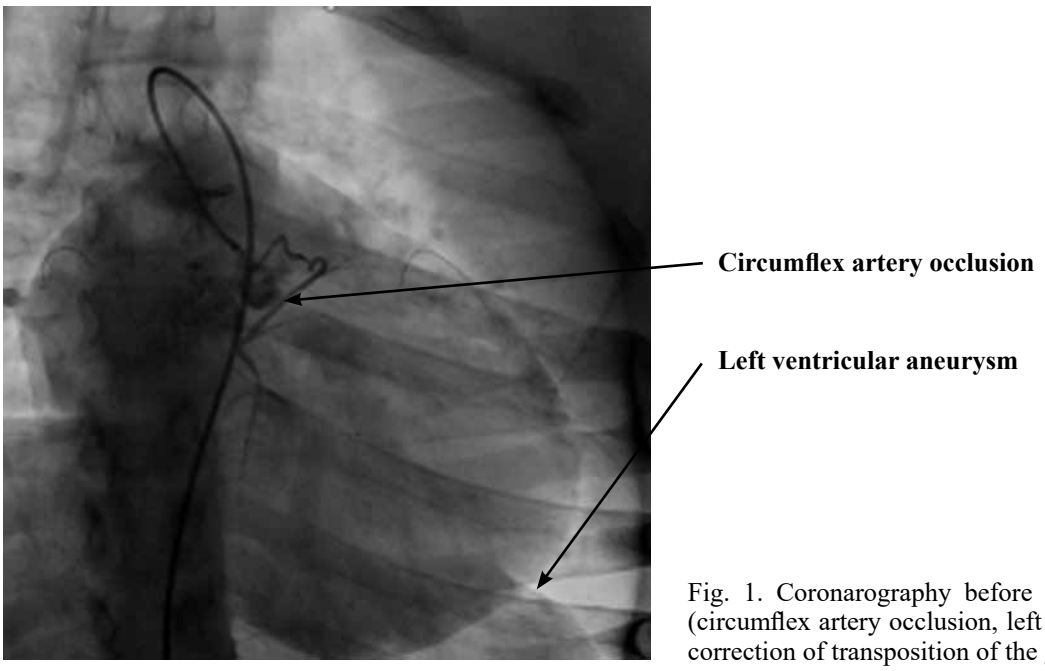

Fig. 1. Coronarography before HeartMate 3 implantation (circumflex artery occlusion, left ventricular aneurysm after correction of transposition of the great arteries)

Contrast-enhanced chest CT scan was performed in all patients to assess the possibility of placing a VAD in the pericardial cavity. A program was used to build a 3D model, which allowed to determine the location of the device – either completely in the pericardial cavity or partially in the left pleural cavity (Fig. 2).

This study also allowed to visualize the coronary anatomy more clearly.

Perioperative risk assessment system

Assessment of biochemical and hematological parameters before surgery allowed to prepare the patient more thoroughly for surgical intervention and assess the risk of adverse complications (Table 4). If these indicators went beyond the target values, the patient was awarded scores. Cardiotonic agents in the preoperative period, use of circulatory assist devices and duration of mechanical ventilation should also be considered, which is also reflected in the scores that are then summed up after which the risk of VAD implantation is determined.

If the patient scores from 0 to 8 points, VAD implantation risk is low; if from 9 to 16 points – medium risk; from 17 to 19 points – high risk of surgery; if >19 points – the patient's hemodynamic state should be first stabilized, blood parameters normalized, and then return to the question of VAD implantation. In our group, all children had a score of 15, meaning a medium risk of surgery.

Features of surgical interventions

All patients were accessed through median sternotomy. Considering that all children had previously undergone correction of complex heart defects, there was pronounced adhesion process in the pericardial cavity, which required a long and thorough cardiolysis due to the need to isolate not only the anterior surface of the

Table 2

Preoperative echocardiographic data

Indicators:	Patient 1*	Patient 2**	Patient 3	Patient 4***
Left ventricle:				
EDV (ml)	223	94	231	246
EDD (cm)	6.6	4.4	5.0	6.9
EF (%)	18	22–24	32–35	25–30
Right ventricle:				
EF/TAPSE		32/8	38/9	40/11
Mitral valve:				
regurgitation	No	Peak gradient 18 mmHg	No	No
stenosis	No	Grade 1	No	No
Tricuspid valve:				
regurgitation	No	Peak gradient 5 mmHg	Grade 1	Grade 1
stenosis	No	Grade 1	No	No
Aortic valve:				
regurgitation	No	No	Grade 1	Peak gradient 50 mmHg
stenosis	No	No	No	Grade 2
Pulmonary artery valve:				
regurgitation	No	No	Grade 1	Grade 1
stenosis	No	No	No	No

Note. EDV, end-diastolic volume; EDD, end-diastolic diameter; EF, ejection fraction; TAPSE, tricuspid annular plane systolic excursion; *, single right ventricle; **, mitral valve implantation and tricuspid valve implantation; ***, aortic valve implantation.

Table 3

Pre-operative evaluation of central hemodynamics in patients

Indicators	Patient 1	Patient 2	Patient 3	Patient 4
Pulmonary artery pressure:				
Systole	30	30	18	27
Diastole	23	12	11	18
Mean	24	20	14	15
PCWP (mmHg)	16	7	10	10
Cardiac index (l/min/m^2)	1.79	3.55	3.06	3.93
Stroke index (ml/m^2)	15.2	61.3	34	59.5
Total peripheral vascular resistance (DSm^2/cm^5)	3618	1867	1753	1140
Transpulmonary gradient (mmHg)		6	4	8

Note. PCWP, pulmonary capillary wedge pressure.

heart and great vessels, but also the left ventricular apex and posterior wall. In two patients, the pericardium was incised in the region of the left ventricular apex with a 2 cm longitudinal incision in order to increase the bed for implantation of the apical pump of the system. The VAD was then connected by separate cannulation of the vena cava and ascending aorta. The heart was dislocated from the wound and the apical cannula implantation site was determined under transesophageal echocardiography (TEE) at the left ventricular apex in the avascular zone. Placing the VAD correctly at its location is a prerequisite for adequate functioning of the VAD; it should clearly face the mitral valve and be equidistant from the interventricular septum and basal parts of the left ventricle. A "coupling" was fixed to the left ventricular myocardium to connect it to the inflow part of VAD. A hole in the LV apex was formed with a ring knife 12 for implantation of the apical cannula. The HeartMate 3 system was implanted with reinforced conduit #12 fixed to the left ventricle using a "coupling" (Fig. 3).

The conduit was placed in the pericardial cavity along the free wall of the right ventricle and right atrium. It is necessary to carefully measure the length of the conduit to avoid its kinks and lack of tension as the child grows (possible compression of the right heart chambers). The anterior wall of the ascending aorta, to which the distal end of the conduit was anastomosed end-to-side with

Table 4

Biochemical and hematologic parameters and risk factor assessment

Indicators (points)	Patient 1	Patient 2	Patient 3	Patient 4
Hemoglobin	95	98	101	107
Platelets ≤148 × 10^3 (7)	276	245	251	450
Albumin ≤33 g/l (5)	37	41	40	35
Hematocrit ≤34% (2)	30	31	28	30
Urea >51 (2)	2.7	3	7.8	7.2
INR >1.1 (4)	2.5	1.86	1.93	2.08
AST 45 (2)	29	20	26	42
Mean PA pressure ≤25 mmHg (3)	24	20	14	15
Vasodilators (4)	Yes	Yes	Yes	Yes
Artificial ventilation	–	–	–	–
ECMO/IABP	–	–	–	–
Cardiotonic therapy (2)	Yes	Yes	Yes	Yes

Note. INR, international normalized ratio; AST, aspartate aminotransferase; PA, pulmonary artery; ECMO, extracorporeal membrane oxygenation; IABP, intra-aortic balloon pump.

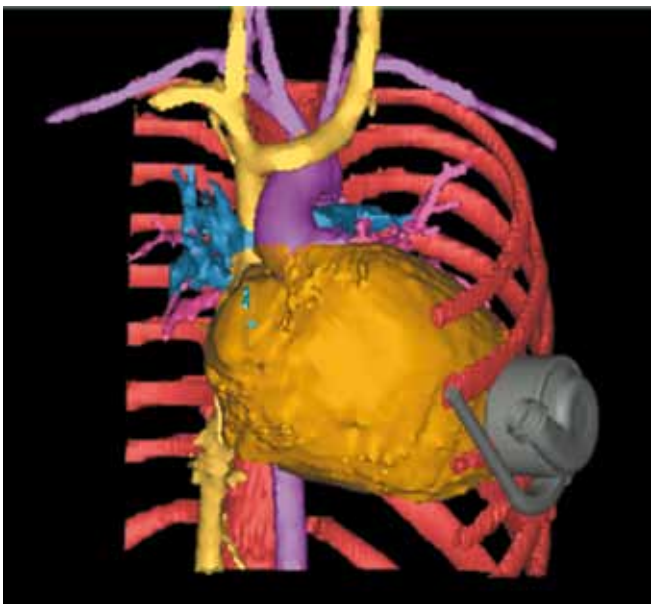

Fig. 2. 3D modeling of the location of the ventricular assist device in the pericardial cavity (location of HeartMate 3 in the pericardial cavity)

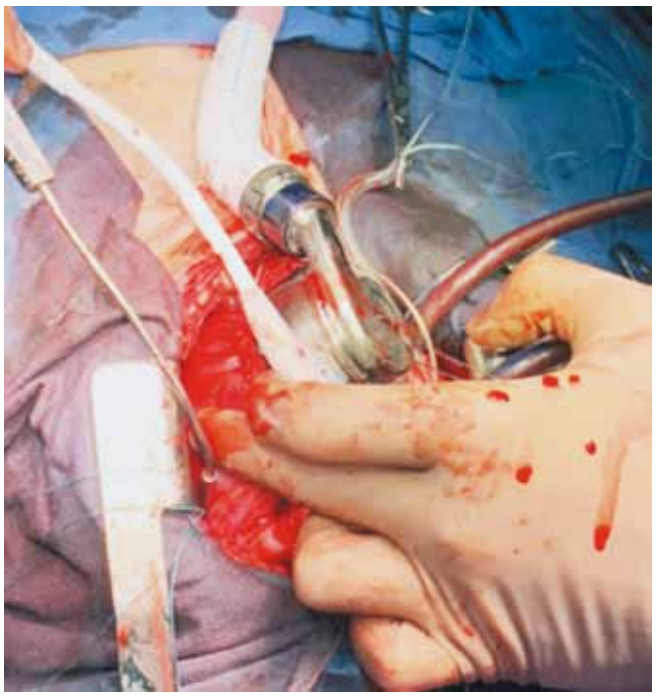

Fig. 3. Implantation of HeartMate 3

a continuous suture using Promilene 5-0 thread, was clamped at the anterior wall of the ascending aorta.

The pump drive cable was led through the contraincision to the anterolateral surface of the abdominal wall on the left side, the cord was placed in the subcutaneous fatty tissue in an S-shape. The HeartMate 3 pump was connected to the system unit through the cable and started working at 2000 rpm, maintaining a flow of 300–500 mL per minute. A control TEE was performed, which visualized the correct position of the apical cannula, absence of blood flow obstruction, and assessed the contractile and pumping function of the right and left ventricles.

When hemodynamic parameters were satisfactory, artificial circulation ended with increased pump operation.

In one patient, implantation of HeartMate 3 left VAD was combined with aortic valve reprotection. After aortic clamping and administration of cardioplegic solution Custodiol, the ascending aorta was opened by a transverse incision 2 cm above the fibrous ring. On revision of the aortic prosthesis, there was pannus buildup with wedging of one leaflet. The valve was excised, after which a sutureless aortic valve PERCEVAL 21 mm was implanted in the aortic valve position (Fig. 4).

Early postoperative period

The postoperative period was uneventful in three patients (75%). Length of hospital stay, hemodynamic, hematological, biochemical parameters, as well as the performance of HeartMate 3 left VAD are presented in Table 5.

The follow-up period in the long-term period was 1 year, 3 months, 4 months and 3 months, respectively. During this period, children's tolerance to physical activity increased, they fully adapted socially, and attended school.

Fig. 4. Implantation of aortic heart valve Perceval

In one patient with initially reduced right ventricular ejection fraction (preoperative RVEF 32%), refractory severe right ventricular failure developed intraoperatively (RVEF 18%, displacement of the interventricular septum into the left ventricular cavity, central venous pressure (CVP) 18 mmHg). It was decided to intraoperatively perform right ventricular bypass according to the "right femoral vein/pulmonary artery" scheme using a centrifuge pump without an oxygenator. A Medtronic 19 Fr venous cannula was placed into the right femoral vein by puncture. A 10-mm-diameter vascular prosthesis was sutured to the pulmonary artery trunk, which was brought to the anterior chest wall through the 5th intercostal space. Medtronic 17 Fr was inserted through the vascular prosthesis into the pulmonary artery trunk (Fig. 5).

The use of the right ventricular bypass lasted for 8 days. The patient's hemodynamic parameters, EchoCG data, right ventricular bypass performance and HeartMate 3 left VAD performance are presented in Table 6.

The right ventricular bypass system was removed on day 8 after surgery.

Artificial ventilation lasted for 20 days and 14 hours. The child was transferred to the ward on day 24. The patient completed a full course of drug and rehabilitation therapy (considering his asthenization). The follow-up period after the operation was 1 year, 1 month. The child gained weight, his tolerance to physical activity increased, and he fully adapted socially.

DISCUSSION

MSC is currently the main method of treatment for ESHF in adults [2, 3]. With the improved experience and success of VADs in the adult population, adoption and adaptation of these technologies for use in the pediatric population were the next steps.

CHD is the most common diagnosis in pediatric patients hospitalized for HF [4]. This patient cohort has a high lifetime risk of HF, especially in older adults and adults whose CHD was not corrected during the neonatal period or early childhood, and according to various authors, 10–20% of them require HT because of residual hemodynamic or anatomical anomalies and consequences of the course of the defect or previous surgery [5, 6]. This includes both pediatric CHD patients who have undergone surgery for CHD and are now older, as well as adult patients (over 19 years of age) diagnosed with CHD for the first time or who are under observation and receiving drug therapy. In a 2018 analysis, 25% of patients requiring MSC were diagnosed with CHD, compared to the previous PediMACs report from 2016, where 16–17.5% of patients with VAD had CHD at baseline [7]. Typically, these patients will be younger, smaller in stature, and have had previous cardiac surgery com-

pared to patients with cardiomyopathy, myocarditis, or complex cardiac arrhythmias [7].

Treatment of pediatric ESHF has improved significantly over the past decade with increased use of VADs [8]. According to the International Society for Heart and Lung Transplantation (ISHLT) International Thoracic Organ Transplant (TTX) Registry, more than one third of pediatric recipients are currently on VAD [9]. As the

Table 5

Postoperative period in 3 patients after HeartMate 3 implantation

Indicators	Patient 1	Patient 2	Patient 3
Mechanical ventilation duration (hours)	14	91	21
Cardiotonic therapy:	Levosimendan, 0.2 mcg/kg/min	Levosimendan, 0.2 mcg/kg/min; Dobutamine, 3.5 mcg/kg/min	Norepinephrine, 0.05 mcg/kg/min; Dobutamine, 4 mcg/kg/min
Length of hospital stay (days)	28	32	28
Hemoglobin	86	101	89
Hematocrit	27	28	26
Platelets	276	251	450
Albumin	40	41	35
Urea	2.7	4	7.2
AST	20	21	42
INR	2.8	2.7	2.2
Pump flow (liters)	5.3	3.4	3.8
Pump speed (rpm)	6050	4850	5100
Power	4.7	3.2	3.5
Pulse index	2.1	5.5	6.2
RVEF (%)	24	38	48
LVEF (%)	–	34	41
mAP (mmHg)	72	69	74
Heart rate	102	98	94
CVP (mmHg)	11	10	12
Prosthetic aortic valve function: Peak gradient Mean gradient	 –	 –	 8 3

Note. AST, aspartate aminotransferase; INR, international normalized ratio; RVEF, right ventricular ejection fraction; LVEF, left ventricular ejection fraction; mAP, mean arterial pressure; CVP, central venous pressure.

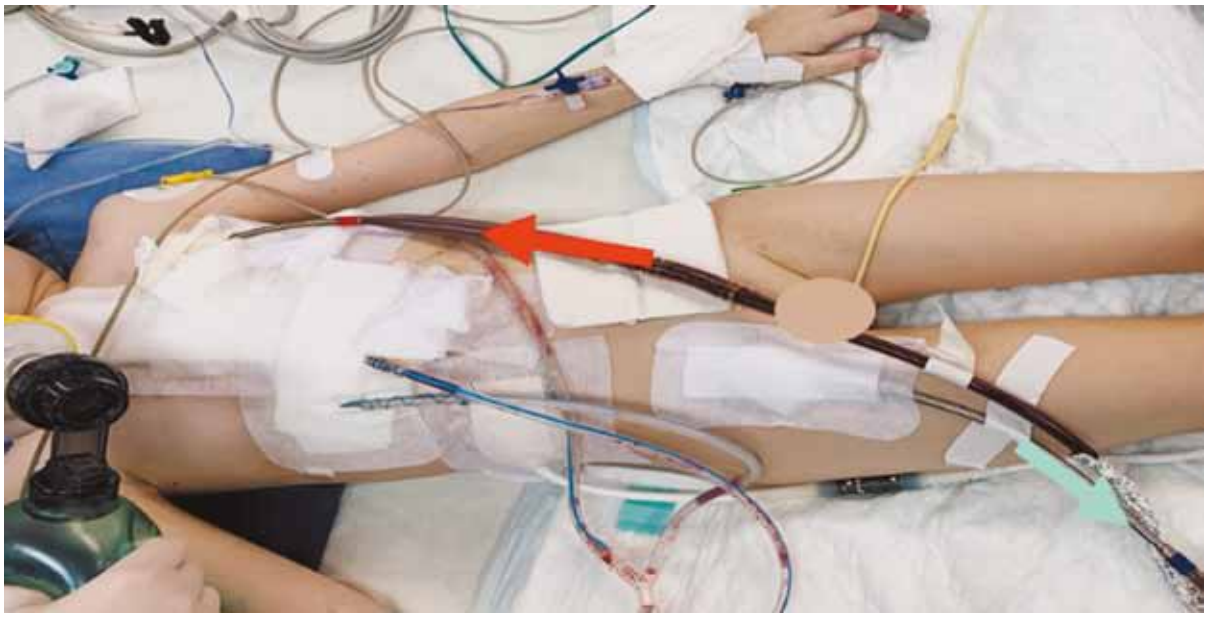

Fig. 5. Use of right ventricular assist device in the early postoperative period in a patient with right-sided heart failure after HeartMate 3 implantation

use of VADs to treat pediatric HF has increased, waiting list mortality has been halved since VAD implantation began [10]. Previously, strategies for mechanical support in the pediatric population were limited to venoarterial extracorporeal membrane oxygenation (VA-ECMO) or paracorporeal pulsatile-flow VADs (Berlin Heart), but continuous-flow VADs are now preferred due to improved survival and reduced side effects [2, 11–13]. Their use in pediatrics has been limited due to size constraints [14, 15]. Currently, long-term circulatory assist systems in the pediatric population are implanted for cardiomyopathy, but usage in the CHD population, especially in patients with complex congenital defects, is increasing [7, 16].

In the U.S. and European medical community, there are registries that collect aggregate data from clinics and summarize information on the use of long-term circulatory support systems, describing their outcomes, patient management tactics, and potential complications.

The Pediatric Interagency Registry for Mechanical Circulatory Support in North America (PediMACS) was created in 2012 and collects prospective data from pediatric VAD patients less than 19 years of age. PediMACS currently includes close to 600 patients with close to 750 implanted devices [13].

The European Registry for Patients with Mechanical Circulatory Support (EUROMACS) is a registry that collects data on both adult and pediatric patients on MCS across Europe [17]. As part of this registry, Pedi-EUROMACS operates to summarize and evaluate all clinical data collected from the pediatric population on MCS. In their second report, Pedi-EUROMACS outlined their findings since inception. A total of 353 patients with 398 implantable devices were included in the most recent analysis. One of their key findings was that survival in patients >10 kg was not significantly different between paracorporeal (pulsatile) and VADs (continuous) devices, indicating that perhaps VAD type is not a major risk factor for worse outcomes, and perhaps patient characteristics may play a more significant role, as patients with a BSA <1 m^2 had higher mortality than patients with a BSA >1 m^2, regardless of flow type.

Advanced Cardiac Therapies Improving Outcomes Network (ACTION) is a multicenter learning health system focused on improving critical pediatric heart failure. Utilizing ACTION, a study was conducted that outlined the outcomes of 35 pediatric and adult patients with complex congenital heart disease who were implanted with HeartMate 3 [13]. Most patients with CHD had Fontan circulation. This study provided the first evidence that HeartMate 3 can be used effectively in pediatric patients.

In our clinical practice, we implanted the HeartMate 3 system in four patients who had previously undergone several surgeries for complex congenital heart defects. While VAD implantation in patients who underwent the Fontan procedure has been described in reports, there are no such reports for implantation after correction of complex congenital anomalies [18].

The patient who underwent definitive correction of DORV and replacement of both atrioventricular valves with implanted CRT-P was resistant to drug therapy. VAD implantation was the only option to alleviate the patient's condition.

Patients after correction of transposition of the great arteries (TGA) and aortic valve replacement with left coronary artery (LCA) and left anterior descending (LAD) artery stenting had reduced left ventricular ejection fraction (LVEF) and were at risk of sudden death from impaired coronary blood flow and risk of developing complex cardiac arrhythmias.

Sutureless aortic valve PERCEVAL was chosen due to the ability to reduce the duration of artificial circulation, reduce the traumatic nature of the operation and prevent adverse events.

Bakulev Center for Cardiovascular Surgery has experience in implantation of various long-term circulatory support systems as a bridge to transplantation in children. Three patients with dilated cardiomyopathy,

Table 6

Postoperative period in a patient after implantation of HeartMate 3 right ventricular assist device

Indicators	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
VAD:								
Flow	2.9	2.8	2.5	2.7	2.4	2.9	2.7	2.9
Pump speed	4800	4800	4800	4800	4800	4800	4800	4800
Pulse index	2.7	2.9	2.9	3.1	2.4	2.4	2.6	2.5
Power	5.0	4.8	4.3	4.7	4.2	4.8	5.0	4.3
RVAD:								
Flow (liter)	1.8	1.8	1.8	1.6	1.4	1.2	1.0	0.8
Pump speed (rpm)	4500	4600	4650	4200	3750	2860	2320	1900
mAP	64	68	71	70	67	74	68	70
CVP (mmHg)	4	11	13	9	7	12	10	10
LVEF	32	31	30	29	31	30	28	32
RVEF	18	32	37	40	47	45	46	51

Note. VAD, ventricular assist device; RVAD, right ventricular assist device; mAP, mean arterial pressure; CVP; central venous pressure; LVEF, left ventricular ejection fraction; RVEF, left ventricular ejection fraction.

postpartum cardiomyopathy and Uhl's anomaly, aged 16 to 17.5 years, and a BSA >1.5 m^2, were implanted with VAD Berlin Heart Excor and NasaDeBakey. They underwent HT within 8 months after implantation. One patient with dilated cardiomyopathy aged 7 years and a BSA <1.5 m^2 was implanted with the Berlin Heart Excor system. Due to the complex system for monitoring VAD and its large size, the child had to remain in the hospital under observation for 493 days.

Considering our experience with implantation of various circulatory assist systems, we concluded that the implantable HeartMate 3 system is compact, safe for small patients with minimal risk of possible adverse events.

CONCLUSION

HeartMate 3 can be implanted in patients with a body weight ≥21 kg and BSA ≥0.88 m^2. Children have increased exercise tolerance (they can walk for 2–3 hours), they fully adapt socially and can attend school. To prevent adverse events in the early and late period after VAD implantation, it is necessary to carefully approach the diagnosis and treatment of patients in the preoperative period.

The authors declare no conflict of interest.

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MECHANICAL CIRCULATORY SUPPORT DEVICES FOR PATIENTS WITH SMALL ANTHROPOMETRIC INDICATORS

O.Yu. Esipova[1], A.S. Esipov[2], A.P. Kuleshov[1], N.V. Grudinin[1]

[1] Shumakov National Medical Research Center of Transplantology and Artificial Organs, Moscow, Russian Federation

[2] Vishnevsky Central Military Clinical Hospital, Krasnogorsk, Russian Federation

Mechanical circulatory support (MCS) devices, designed specifically for patients with small anthropometric parameters, are now emerging. A detailed systematic literature review of existing systems for long-term circulatory support in this patient cohort was conducted. Circulatory support devices and their main technical and biological characteristics were studied in detail. Despite significant scientific and technological progress, there is still no technology for creating an assist pump to support patients with small body surface area (BSA), given the wide range of patient sizes, increased cardiovascular demand due to growth, as well as anatomical and physiological heterogeneity of congenital heart disease.

Keywords: mechanical circulatory support, body surface area, axial pump, centrifugal pump.

Severe cardiovascular disease in adults and children is the leading cause of death worldwide, claiming 17.9 million lives annually [1]. Heart failure (HF) is a consequence of severe heart disease in which the heart muscle is unable to pump blood to provide adequate end-organ perfusion. HF affects the quality of life of people around the world, affecting 64 million adults and children each year [2]. The reported incidence of HF in children worldwide is 0.97% to 7.4% per 100,000 children [3], and most of them require immediate surgical intervention [4]. Infants with complex congenital heart defects may require multiple open-heart surgeries to establish proper cardiovascular anatomy and physiology [5–7]. These complex congenital heart disease (CHD) cases require continuous clinical follow-up throughout the patient's life, as this cohort of patients is at a higher risk of developing premature congestive HF. Heart disease can also manifest with developmental delays, including neurological impairment and growth retardation [8].

Pharmacologic drug therapy slows progression to end-stage HF. Heart transplantation is the gold standard of medical care, but the number of patients requiring transplantation keeps on exceeding the number of available donor organs every year. In pediatric transplantation, a difficult point is the selection of donor-recipient pairs due to anthropometric features of young patients. Statistically, 74% of children receive a donor organ within 90 days of being placed on the waiting list, but the mortality rate among those still waiting ranges from 5% to 39% worldwide [9]. There remains a high clinical need to develop safe and effective mechanical circulatory support (MCS) devices for these HF patients for use as intermediate therapy or long-term chronic disease management.

MCS devices are used as a bridge to transplantation, a bridge to recovery and permanently (or as a definitive treatment option without the possibility of heart transplantation) (bridge to destination). Analysis of the UNOS database shows that this technology has led to a 50% reduction in waiting list mortality [10]. Despite this progress, there remain important aspects in which these devices can be improved. This review presents published data on MCS devices created and under development for patients with small body surface area (BSA) in order to assess progress and provide an informed vision for the development of this industry. Table 1 summarizes the major devices developed and their key technical specifications.

MAIN CRITERIA WHEN DESIGNING MCS DEVICES

According to the characteristics of the devices presented in Table 1, the main design criteria identified were:

1) pulsatile or continuous flow;
2) acute or chronic circulatory support;
3) anatomical location in the patient's abdominal cavity;
4) blood rheology;
5) dynamic pressure and blood flow requirements in patients of different ages.

Pulsatile or continuous flow

Recent clinical evidence in adults show that chronic continuous flow disorders with reduced pulse pressure can lead to harmful side effects and adversely affect outcomes [11–13]. However, it has also been found that

Corresponding author: Olga Esipova. Address: 1, Shchukinskaya str., Moscow, 123182, Russian Federation. Phone: (925) 190-96-14. E-mail: olgadmitrieva2008@yandex.ru

continuous blood flow conditions are well tolerated for short-term circulatory support and may provide better performance than pulsatile flow devices. Currently, there is still a poor understanding of how small anthropometric patients tolerate long-term implantation of MCS devices whether with pulsatile or continuous flow. Consequently, the development of ventricular support devices that can generate continuous and pulsatile blood flows is an urgent problem in modern medicine [14, 15].

Acute and chronic needs for MCS devices

Of the cases reported in PediMACS, 19% of patients received short-term or emergency support via MCS devices [16]. Treatment outcomes for these patients were better compared to extracorporeal membrane oxygenation (ECMO) techniques, resulting in increased implementation rate, and the mean time to use short-term MCS increased to 19 days [17]. Some MCS devices designed for short-term use (Thoratec Centrimag & Pedimag Blood Pump, Thoratec Corporation, USA) were used as long-acting devices and showed favorable outcomes. The disadvantage of these systems was that they limited patient mobility and prolonged the length of stay in specialized medical centers.

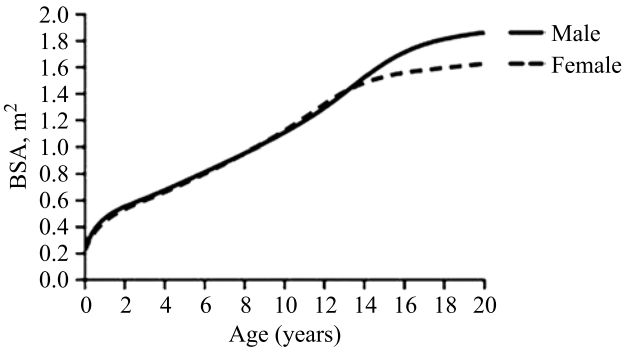

Fig. 1. Change in BSA in healthy individuals aged 0 to 20 years

Anatomical location of MCS devices in the patient's abdominal cavity

In patients with small anthropometrics, there are several requirements for device placement: in terms of anatomical positioning and connection of the inlet and outlet cannula. The BSA for patients can be easily calculated from body weight and height:

$$\text{BSA}\left[\text{m}^2\right] = \sqrt{\frac{\text{Height (cm)} \times \text{Weight (kg)}}{3600}}.$$

The BSA of newborn patients averages 10% of the BSA of a young adult, and it increases dramatically as the infant grows and develops (Fig. 1) [18].

Consequently, anatomical fitting of an MCS device for patients with low BSA is both challenging and ne-

Table 1

Key technical design specifications of the developed MCS devices

Device	Country	Working fluid flow (L/min)	System pressure (mmHg)	Total length (mm)	Body surface area (m^2)	Market position
DON-3 (10 mL)	Russia	1–3	80	60	0.62–1.1	–
Berlin Heart Excor (10 mL)	Germany	0.6–1	225	Paracorporeal system	0.2–0.33	FDA approved for clinical use in pediatric patients
Berlin Heart Excor (25 mL)	Germany	1.3–2.2	175	Paracorporeal system	0.33–0.5	FDA approved for clinical use in pediatric patients
Berlin Heart Excor (30 mL)	Germany	1.3–3	175	Paracorporeal system	0.6–1	FDA approved for clinical use in pediatric patients
Berlin Heart Excor (50 mL)	Germany	3–5.2	175	Paracorporeal system	1–1.7	FDA approved for clinical use in pediatric patients
Berlin Heart Excor (60 mL)	Germany	3.6–6	200	Paracorporeal system	1.2–2	FDA approved for clinical use in pediatric patients
DeltaStream DP3 VAD (240 mL)	Germany	0–8	240		0.18–0.61	–
HeartMate 3 (280 mL)	USA	2.5–10	280	50.3	>1.2	FDA approved for clinical use in pediatric patients
HeartWare HVAD (135 mL)	USA	2–10	–	49	>1.2	FDA approved. But no longer available on the market
Jarvik Infant VAD (60 mL)	USA	0.5–3	–	11	>0.4	
PediaFlow VAD (155 mL)	USA	0.3–2	–	28	0.2–0.8	
Penn State Infant VAD (12–14 mL)	USA	0–1.6	–		>0.5	

cessary. The design of paracorporeal devices also needs to consider the patient's anatomy, which determines which inlet and outlet cannulas would be suitable. For example, the Berlin Heart Excor (Berlin Heart GmbH, Germany) paracorporeal circulatory assist (CA) system has a size range of exit cannulas with diameters of 3, 6, 9, and 12 mm [19–21]. An increase in BSA with age generally suggests that devices designed for younger patients should be suitable for older patients as well, while factoring in the device size for all cohorts.

Blood rheology in patients with low BSA

Patients of different ages differ in blood rheological properties [22, 23]. Hematocrit, which affects blood viscosity, is highest in newborns and rapidly decreases to a steady level as adolescence is reached. Congenital or acquired heart defects in patients with low BSA also affect hematocrit levels, and blood rheological properties affect fluid dynamics, especially in low phase shift CA pumps [24].

Dynamic pressure and blood flow requirements in patients of different ages

Young patients experience increased cardiac volume during growth and development, and hence the dynamic pressure requirements of an MCS assist device change. Cardiac output in children doubles from birth to 1 year of age and doubles again by 10 years of age (Fig. 2).

The size of an MCS device determines the ability to generate a wide range of pressures and flow rates at acceptable shear stress [25]. This poses some challenges related to external design, as device size and hence anatomical positioning is inversely related to pressure and capacity. For long-term mechanical support, patients with small anthropometrics may require replacement of the MCS system with a new one to adapt to the patient's height. The versatility of the design can be utilized to integrate multiple pumps into a single device to increase the operating range of flow and pressure characteristics.

EXTRACORPOREAL CA SYSTEMS AND LVAD SYSTEMS

MCS devices and the challenges faced by physicians and patients using this type of CA systems were reviewed. But two categories of MCS devices are more often used in clinical practice: extracorporeal circulatory support systems and ventricular assist devices.

Extracorporeal circulatory support systems for patients with small anthropometric indicators

One of the earliest technologies and still the most sought after for patients with small BSA is ECMO [26–28]. The ECMO circuit is connected to the patient either by veno-arterial cannulation (via the femoral artery and vein) or by veno-venous cannulation (via the right atrium or jugular vein). The ECMO device circulates and oxygenates the blood, replacing both heart and lung function.

This system is designed for short-term support and requires immobilization and often sedation [29]. Therefore, extracorporeal ventricular assist device (VAD) systems have begun to compete with ECMO.

When the performances of ECMO and VAD were compared for patients with small anthropometric indices, it was found that those who received extracorporeal pump support showed improved outcomes. Waitlist mortality also decreased from 38% (ECMO) to 13% (VAD), while post-transplant survival increased from 80% to 92% in patients receiving VAD support instead of ECMO [30].

Extracorporeal and paracorporeal devices such as Berlin Heart EXCOR (Berlin Heart, Germany), PediMag

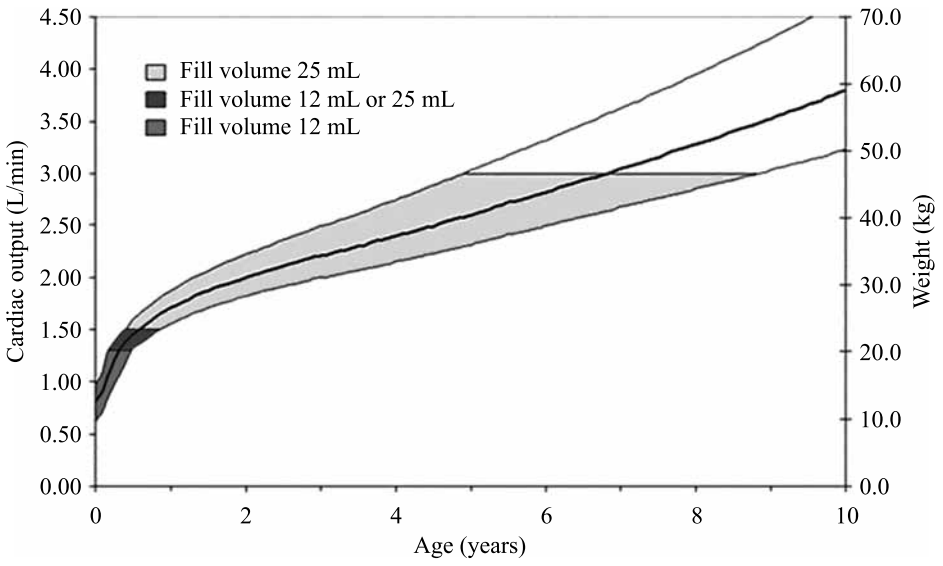

Fig. 2. Cardiac output requirements for children aged 0 to 10 years

(Abbott Laboratories, Illinois, USA), Jostra RotaFlow (Maquet, Germany) and TandemHeart (CardiacAssist, Inc., USA) have been used to support cardiac ventricular function in the short-term without oxygenation. These pumps compare favorably with ECMO for short-term treatment.

Since these devices are placed outside the body, the issue of placing pumps in the patient's abdominal area is no more there. In addition, transferring the patient to another device is less complicated with extracorporeal/paracorporeal devices. However, for long-term support, these MCS devices face several challenges. These designs limit patient mobility, increase thromboembolic complications, increase neurologic risks compared to implantable designs, and limit hospital discharge due to home care challenges [31].

Ventricular assist devices

At their core, VADs increase cardiac output by assisting the left or right ventricle. In 2004, the National Institutes of Health's National Heart, Lung, and Blood Institute (NHLBI) established the Pediatric Ventricular Assist Device Program. These programs have facilitated the development and implementation of implantable devices for patients with small BSA [32–33].

Projects under the program included the development of the following devices:

- An implantable mixed-flow device (PediaFlow VAD) [34];
- An implantable mixed-flow VAD that can be maintained both intravascularly and extravascularly, depending on patient age and size (PediPump) [35];
- Ension's Pediatric Cardiopulmonary Assist System (pCAS) for patients with small anthropometric parameters [36];
- Axial pump (Infant Jarvik) [37];
- Pulsatile flow system (Penn State pediatric VAD) [38].

Other devices have been developed in parallel with this development program, some of which have been approved for clinical use in patients with small BSA.

Clinically approved MCS devices

Berlin Heart EXCOR (Berlin Heart, Germany) is one of the first systems approved by the U.S. Food and Drug Administration (FDA) (Fig. 3) [39–40].

It is a flexible diaphragm pump with chamber fill volumes ranging from 10 to 80 mL and flow rates from 0.4 to 5 L/min. All of them provide sufficient pressure rise to support patients with small BSA [41].

Two other FDA-approved VADs are the implantable HeartMate 3 LVAD (Abbott Laboratories, USA) (Fig. 4), which is designed for patients with a BSA >0.7 m^2 for extracorporeal support and 1.4 m^2 for implantable support [42], and the extracorporeal Abiomed BVS 5000 (Abbott Laboratories, USA).

Fig. 3. Sizes of the Berlin Heart EXCOR extracorporeal pumps (10 to 80 mL fill volume)

Fig. 4. Implantable HeartMate 3 LVAD

The HeartMate 3 adult assist pump (Abbott Laboratories, USA) received FDA regulatory approval for use in patients with small anthropometric indices with progressive right ventricular dysfunction in December 2020 [43]. This fully magnetic suspension pump has demonstrated very good outcomes in adult patients (2-year survival rate is 79%) and has received approval for use in patients with BSA >1.2 m^2.

Implantable heart pump HVAD (Medtronic, USA) was approved by the FDA in 2012 for adult patients with

large BSA. However, after a retrospective analysis of this pump in 14 patients with low BSA, it was rejected for use in this patient cohort. Production of these pumps was suspended in 2021 due to the high incidence of adverse neurologic events [44].

As part of the MCS device development program described earlier, Jarvik Heart has developed a VAD that can be used in patients with low BSA (Fig. 5) [45]. Infant VAD Jarvik can generate flow from 0.27 to 3 L/min, Child VAD Jarvik produces flow from 0.5 to 3 L/min.

The devices received FDA approval for clinical trials in 2012, but were subsequently recalled as of late 2018, and were no longer in clinical use by May 2020 due to connector issues on external cables [46].

The developers of the PediaFlow axial pump (HeartWare International, USA) (Fig. 6) have continued independent development to date, despite no FDA approval. The most recently published fourth-generation version of the device is a compact design (17 mm in diameter and 50 mm in length) that can support patients weighing up to 3 kg and can deliver blood flow rates from 0.5 L/min [47].

Fig. 5. Axial flow pumps: Child VAD Jarvik (left) and Infant VAD Jarvik (right)

Fig. 6. Axial-flow pump PediaFlow (HeartWare International, USA)

MCS devices for patients with small anthropometric parameters are under development

DeltaStream DP3 (Xenios AG., Germany)

The DeltaStream DP3 is a diagonal pump that combines axial and centrifugal pumps to pump blood. This extracorporeal device easily generates the required pressure (240 mmHg increase in systemic pressure) and the required flow rate of up to 8 L/min. In vitro studies have shown that this device works without interruptions in patients with BSA from 0.18 to 0.61 m^2 [48].

DON-3 (Russia)

DON-3 is the first experience in creating a domestic VAD. This MCS device is an axial pump. The development was brought to the stage of experimental studies on animals (sheep). The pump provides operating pressure of up to 135 mmHg with a liquid flow range 1–3 L/min. The design of the development is relatively compact: diameter 25 mm, length 60 mm. Results from animal tests show promising results [49–51].

Drexel Dragon 1S & 1P (Drexel University, USA)

Drexel University is developing hybrid continuous-flow MCS devices in which a magnetically suspended axial pump and a centrifugal pump are combined to increase the active operating time of the device [52]. There are two design concepts: the axial pump is placed in front of the centrifugal pump, and a parallel concept: the axial and centrifugal part of the pump are separated into two separate units. A pump-to-pump switching technology was created to control this unit. The maximum system pressure rise during initial testing on a hydrodynamic bench is 120 mmHg with a flow range of 1 to 5 L/min. Development of this pump is actively continuing.

iCor VAD (Xenios AG., Germany)

The iCor pump (Xenios AG., Germany) is a paracorporeal centrifugal pump. Initial studies of the flow and pressure characteristics of the pump have been performed and have shown overpressurization of more than 100 mmHg at flow rates ranging from 0.2 to 1.8 L/min. The pump continues to be improved and tested.

NIPRO VAD (NIPRO Medical Corporation, USA)

NIPRO VAD (NIPRO Medical Corporation, USA) is another paracorporeal pulsatile VAD. The flow rate is 2–4 L/min at a maximum pressure of 150 mmHg, at which the device can provide pulsatile flow at a rate of 50–130 beats per minute. Bench tests of this pump have been performed and have shown low levels of damage to blood cells [53, 54].

Penn State Infant VAD (Penn State University, USA)

The University of Pennsylvania is developing a pump targeting patients with low BSA. The VAD under development is a pulsatile paracorporeal device with a

valve and pneumatic actuator, a concept derived from the Thoratec pneumatic VAD. It is a small pump with an operating filling volume of 12–14 mL. Animal studies have shown that the pump can deliver a flow rate of 1.6 L/min. This system is suitable for patients with BSA <0.5 m^2, and there is a low level of blood trauma. Developments are ongoing.

CONCLUSION

The results of this review show that there have been significant progress in the development of MCS devices. New engineering and design capabilities are gradually approaching clinical implementation.

Design constraints for CA devices for patients with small anthropometrics, such as target size, are usually determined based on duration of support (acute or chronic) and clinical goals (extracorporeal placement or implantable option). The choice of cannula connection also depends on the patient's anatomy. Table 2 summarizes the main theoretical parameters that MCS devices require for patients with different BSA [57, 58].

Table 2

Expected performance of MCS devices

Theoretical performance indicators	Target range
System pressure	10–150 mmHg
Fluid flow	0.5–7 L/min
Pulmonary pressure	5–30 mmHg
Pump rotor speed	≤8000 rpm
Shear stress	<170 Pa
System power consumption	<10 W

Using this data, it is possible to assess how well the pumps meet the pressure and flow requirements for patients with small BSA. Most devices cope well with increasing pressure, but there are discrepancies in performance when looking at the reported fluid flow ranges.

Factors such as speed and rotational speed can be altered to create higher blood flow, but it is likely that these alterations can drive the device into non-standard operating states, creating physiologic risks (hemolysis and thrombosis).

The above data show that there is no universal pump yet that satisfies certain design limitations and requirements due to the wide range of patient sizes, higher cardiovascular demand as the body grows, and anatomic and physiologic heterogeneity of congenital heart defects.

Although progress has been made in the development of MCS systems, additional research is needed to inform the broader scientific and medical community and stimulate innovation in medical device technologies for patients with small anthropometrics.

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COMPREHENSIVE NON-INVASIVE EVALUATION OF THE FUNCTIONAL STATUS OF PATIENTS WITH CHRONIC HEART FAILURE

N.N. Koloskova[1], A.Q. Eyyubova[1], A.O. Shevchenko[1–3]

[1] Shumakov National Medical Research Center of Transplantology and Artificial Organs, Moscow, Russian Federation

[2] Sechenov University, Moscow, Russian Federation

[3] Pirogov Russian National Research Medical University, Moscow, Russian Federation

The emergence of new groups of medications used in the treatment of chronic heart failure (CHF) has made it possible to optimize treatment regimens, changing the clinical status and prognosis in this patient cohort. In this regard, the relevance of individual prognostic markers and risk assessment scales for heart failure (HF) is losing its value. The aim of our review is to summarize the currently available evidence on modern methods of evaluating the functional capabilities of the body and exercise tolerance in CHF patients on the background of systolic dysfunction before heart transplantation.

Keywords: heart failure, heart transplantation, cardiopulmonary exercise test, 6-minute walk test, atrial natriuretic peptide, asthenia, waiting list.

With the increasing number of chronic heart failure (CHF) cases [1, 2], timely detection of the moment the disease transits from stable to end stage is crucial for the choice of further treatment tactics and assessment of survival prognosis in this category of patients [3]. To date, various prognostic risk scales have been developed and used in assessing CHF patients [4, 5]. However, statistics has shown that doctors are reluctant to use them in their daily practice, and the scales themselves do not provide complete information on patient survival prognosis [6, 7].

The previously developed Heart Failure Survival Score (HFSS), which was widely used in selection of patients for inclusion in the heart transplant waiting list, is now losing its relevance due to the emergence of new approaches to drug therapy in CHF patients [8]. Today, quadruple therapy is the gold standard treatment for patients with reduced left ventricular ejection fraction (LVEF). The concept of quad therapy includes the use of a combination of the following drug groups: beta-blockers, sodium-glucose co-transporter 2 (SGLT2) inhibitors, mineralocorticoid receptor antagonists, and renin-angiotensin-aldosterone system (RAAS) inhibitors. Large, randomized studies have shown that quadruple therapy significantly reduces the frequency of hospitalizations for decompensated HF and improved the survival prognosis in this patient cohort [9].

Although heart transplantation (HT) remains the only effective curative treatment for end-stage CHF and the waitlist criteria have expanded significantly in recent decades, organ shortages do not fully meet the need for curative treatment of patients with end-stage HF [10]. In this regard, there is a need to develop new approaches for assessing CHF severity and a personalized approach for choosing further treatment tactics.

The aim of our review was to summarize the currently available data regarding modern methods of assessing the functional capacity of the body and exercise tolerance in CHF patients.

Self-assessment of physical condition by the patient and/or by the treating physician depends mainly on what the patient perceives as limitations in their daily activities. The currently widely used New York Heart Association (NYHA) functional classification (FC) allows HF severity to be determined based on patient's complaints (Table 1).

However, this classification is based solely on symptoms and does not include prognostic indicators derived from various functional tests; therefore, it cannot serve as a reliable predictor of adverse events in CHF patients [11–13].

It is important to note that patients with mild symptoms of CHF may have poor survival prognosis despite the apparent perceived well-being of the condition [14].

Cardiopulmonary exercise testing (CPET) remains the gold standard and established tool for assessing the functional capacity in HF. CPET measures variables such as volume of oxygen consumed by the body (VO_2), volume of carbon dioxide produced by the body (VCO_2) and pulmonary ventilation (PV) at rest and during exercise.

Corresponding author: Nadezda Koloskova. Address: 1, Shchukinskaya str., Moscow, 123182, Russian Federation. Phone: (926) 651-40-64. E-mail: nkrasotka@mail.ru

During exercise, the human body can be visualized as an integrated system that provides oxygen (O_2) delivery to the mitochondria for aerobic exercise [15–17]. Oxygen delivery depends on interaction between components of the electron transport chain and its adequate release in working muscles. Table 2 summarizes the main variables obtained during testing, which need further interpretation.

The main parameters derived from CPET

Respiratory exchange ratio (RER) is the ratio between peak VCO_2 production and peak VO_2 consumption. RER values of 1.05–1.15 indicate achievement of a maximal exercise effort in CPET [18].

Workload is the maximum workload a patient can perform during a CPET session. It is measured in watts. Maximum workload >90% predicted indicates that the patient has a high exercise tolerance [19].

Maximum heart rate. CPET is considered complete when the patient reaches a heart rate (HR) ≥90% of the predicted maximum HR, depending on the patient's age. It should be noted that patients under chronotropic medications are not sometimes capable of meeting this criterion. In this case, a maximal exercise may be completed as it is indicated by the interpretation of RER and workload [20].

Peak oxygen consumption (Peak VO_2) is the most important parameter derived from a CPET and at the same time is the gold standard to objectively assess functional limitations in HF patients [21]. Peak VO_2 can be reported as an absolute value (mL/min) or indexed by body weight (mL/min/kg) or as a percentage of predicted value (%) normalized to sex, age, height, and weight measurement [22, 23].

To date, a peak VO_2 <14 mL/kg/min is one of the risk factors for adverse cardiovascular events [24]. Heart transplant guidelines report that HF patients with peak VO_2 ≥12 mL/min/kg (while taking beta-blockers (BB)) or ≥14 mL/kg/min (while discontinuing BB 24 hours before testing) may be safely assigned UNOS status 7 [25, 26].

Anaerobic threshold (AT) gives an idea of exercise tolerance under aerobic conditions. The point of anaerobic metabolism initiation (submaximal exercise) is determined using concentrations of inhaled oxygen and released carbon dioxide during a CPET session [27, 28].

So, CPET is currently the most comprehensive technique for evaluating patients with cardiopulmonary diseases. It may provide supporting information for differential diagnosis in the presence of symptoms such as shortness of breath and poor exercise tolerance between cardiac and respiratory failure and/or physical detraining of the patient. The disadvantages of this method are the need for specialized equipment, training of personnel, and the very high cost of the method itself, which is associated with limited accessibility in most hospitals, as well as the inability of some patients to perform this test due to the severity of their clinical condition. Where CPET cannot be performed, the 6-minute walk test (6MWT) is a simple, inexpensive test that can be performed for risk stratification in CHF patients [29–31].

The 6MWT is a simple test that does not require special equipment and special training of physicians. This test allows assessing the submaximal level of a patient's functional capacity, while walking on a flat hard surface for 6 minutes [32]. Inability to assess the reactions of all organs and systems involved during this test, as in the case of CPET, constitutes a disadvantage [33].

Despite the significant correlation between 6MWT and peak VO_2, this test cannot be considered as an al-

Table 1

New York Heart Association Functional Classification

FC I	No limitation of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation or shortness of breath
FC II	Slight limitation of physical activity. Comfortable at rest. Ordinary physical activity results in fatigue, palpitation, shortness of breath or chest pain
FC III	Marked limitation of physical activity. Comfortable at rest. Less than ordinary activity causes fatigue, palpitation, shortness of breath or chest pain
FC IV	Symptoms of heart failure at rest. Any physical activity causes further discomfort

Table 2

CPET data

Parameters	Expected values at peak exercise
Exercise	
Duration (minutes)	8–12
Workload (% of predicted)	>80
RER	>1.15
Hemodynamic parameters	
Systolic blood pressure (mmHg)	<220
Cardiac cycle (% of predicted HR)	>90
Metabolic indicators	
Peak VO_2 (% of predicted peak VO_2)	>84
Anaerobic threshold (% of predicted VO_2)	>40
Pulse O_2 (%)	>80
VO_2/work (mL/min/W)	9–11
Ventilation	
Respiratory rate (breaths/min)	<60
$PETCO_2$ at baseline (mmHg)	>33
$PETCO_2$ at anaerobic threshold (mmHg) vs. baseline	>3–6
O_2 desaturation (%)	<4
Prognostic	
VE/VCO_2 slope	<34
O_2 recovery slope	>650

ternative to CPET, as the results obtained are not a reliable predictor of changes in peak VO_2 in CHF patients [34–36].

Previous studies have shown that there is an inverse correlation between NYHA FC II–IV and the 6-minute walk distance (6MWD) [37–39]. Table 3 shows the correlation between physical activity parameters assessed via 6MWT, peak VO_2 by CPET and NYHA FC [40].

Several studies have shown that in CHF patients being evaluated for transplantation, a 6MWD <350 meters has a sensitivity of 71% and specificity of 60% for predicting peak VO_2 <14 ml/kg/min during a CPET session [41, 42].

Thus, 6MWT can be used as an alternative to measure the functional status of patients with HF and comorbid pathology, such as chronic obstructive pulmonary disease, when exercise testing is not feasible [43–47].

Modern biomarkers for assessing the severity of CHF and predicting the course of the disease include natriuretic peptides [48, 49].

Recent guidelines from the European Society of Cardiology (ESC) on diagnosis and treatment of heart failure [50] and the American Heart Association (AHA) [51] include brain B-type natriuretic peptide (BNP) and its precursor N-terminal pro b-type natriuretic peptide (NT-proBNP) were included as mandatory markers in HF diagnosis.

Determination of other diagnostic biomarkers, such as inflammatory marker ST2, oxidative stress marker – growth-differentiation factor-15 (GDF-15) – and cardiac remodeling marker – galectin-3 – may be useful in prescribing therapy aimed at HF treatment but are not mandatory in making this diagnosis [51].

In their work, Hogenhuis et al. analyzed a number of indicators of 229 patients who had been admitted for decompensated CHF at the time of hospital discharge. The following parameters were included in the analysis: BNP level 6MWD, LVEF, and NYHA FC. The authors revealed that BNP shows weak correlation to LVEF ($r = –0.29$, $P < 0.01$) and NYHA ($r = 0.20$, $P < 0.01$). There is also no correlation between BNP and 6MWT ($r = –0.01$, $P = 0.87$). Thus, the authors concluded that BNP level reflects the state of cardiac function to a greater extent, whereas 6MWD reflects the functional capacity of the body, and these two indicators represent different aspects of the clinical syndrome of CHF [52].

Table 3

Exercise and oxygen consumption in patients with different functional classes of CHF

NYHA FC	6MWD (m)	Peak VO_2 (mL/min/m^2)
0	>551	>22.1
I	426–550	18.1–22.0
II	301–425	14.1–18.0
III	151–300	10.1–14.0
IV	<150	<10.0

In contrast, a study by Norman et al. conducted a correlation analysis to assess the relationship between BNP levels and peak VO_2 during CPET and LVEF in 22 subjects with compensated HF. The results suggested that plasma BNP levels may be a useful clinical measure for evaluating both global functional capacity and myocardial specific work capacity in individuals with HF [53].

In their study, Kato et al. evaluated peak VO_2 in combination with BNP in 424 potential recipients examined before HT. All patients were divided into three groups depending on peak VO_2. The first, second and third groups included 167, 146, and 111 patients, respectively. Peak VO_2 was >14 mL/min/kg in group 1, 10 to 14 mL/min/kg and <10 mL/min/kg in groups 2 and 3, respectively. The comparison group included 743 recipients after de novo HT. Multivariable analysis revealed that high BNP and low peak VO_2 were independently associated with death, HT, or ventricular assist device (VAD) systems (hazard ratio, 3.5 and 0.6; 95% CI, 1.24–9.23 and 0.03–0.71; $P = 0.02$ and <0.0001, respectively). One-year survival without VAD or without HT in patients with peak VO_2 between 10 and 14 mL/min/kg was comparable to one-year survival after HT. Given these findings, the authors divided the second group into two subgroups based on those with BNP ≥506 pg/mL and those with <506 pg/mL. One-year survival of patients with HF and low BNP levels was comparable to post-HT survival (1 year: 90.8% versus 87.2%; $P = 0.61$), whereas those with BNP ≥506 showed worse VAD-free or HT-free survival (1 year: 79.7%; $P < 0.001$ versus post-HT). It was concluded that a comprehensive evaluation of peak VO_2 during exercise in combination with BNP levels can determine the optimal time frame for inclusion of patients on the HT waiting list [54].

Shyh-Ming Chen et al. analyzed the survival of 377 patients hospitalized for decompensated HF and showed that the risk of adverse events at two years in patients with peak VO_2 of 10.2 mL/kg/min on optimal medical therapy was 20% for the entire cohort of patients. Based on these data, the authors proposed a scheme of an optimized strategy for predicting adverse events, determining the timing and indications for inclusion on the waiting list for HT or continuation of therapy for CHF (Fig. 1) [55].

Current clinical guidelines for the management of HF patients suggest that peak VO_2 obtained during CPET should be used as one of the criteria for determining whether a patient should be listed for HT. In the 1990s, Mancini et al. showed that peak VO_2 of 14.0 mL/kg/min is an indication for inclusion of patients in the HT waitlist [56]. In the 2000s, against the background of the beginning of widespread use of beta-blockers in CHF therapy, the threshold value was reduced to 12.0 mL/kg/min [57]. Recently, due to better survival prognosis

on the background of quad therapy application, the prognostic threshold was reduced to 10.2 mL/kg/min [58].

Recently, increasing importance has been attached to the frailty score in assessing the prognosis of CHF patients and in selecting patients for HT and/or mechanical circulatory support [59, 60].

Yasbanoo Moayedi et al. evaluated the prognostic significance and impact on survival prognosis of frailty in combination with peak VO_2 as a prognostic indicator for assessing the severity of heart failure. Frailty was assessed using modified criteria according to the Fried Frailty Phenotype (FFP) scale. The results were interpreted as frail, prefrail and nonfrail. The study included 201 HF patients. The median follow-up was 17.5 months (11 to 29.2 months). During the follow-up period, overall mortality was 25 patients (12.4%). One-year survival among patients with frail, prefrail and nonfrail were 78%, 94%, and 100%, respectively. Thus, the authors showed that frailty was associated with a twofold increased risk of death (HR 2.01, $P < 0.0001$, 95% CI 1.42–2.84). In a comparative analysis of the effect of this syndrome in combination with peak VO_2 on survival prognosis, it was shown that peak VO_2 <12 mL/kg/min, in combination with frailty, was associated with increased risk of mortality compared with patients with VO_2 >12 mL/kg/min (HR 1.72, $P = 0.006$). It was concluded that the severity of generalized weakness syndrome is one of the risk factors for poor prognosis of 1-year survival in patients with low peak VO_2 [61].

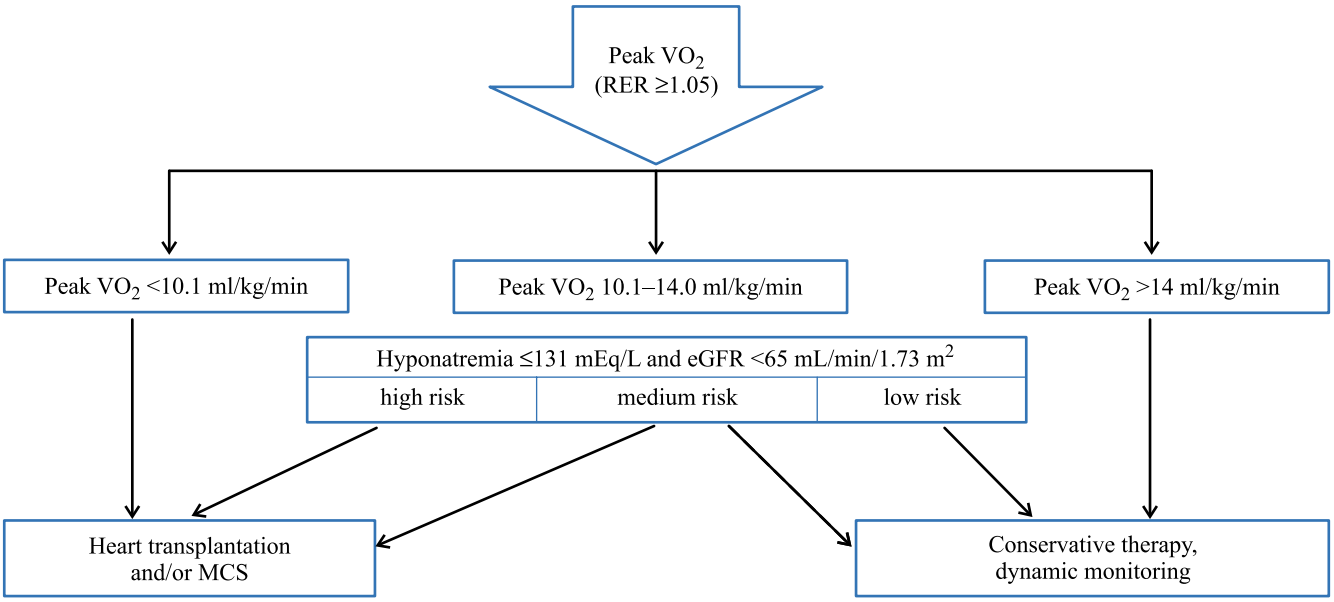

Fig. 1. Optimized strategy for predicting adverse events, timing, and indications for heart transplantation. eGFR, estimated glomerular filtration rate; RER, respiratory exchange ratio; MCS, mechanical circulatory support

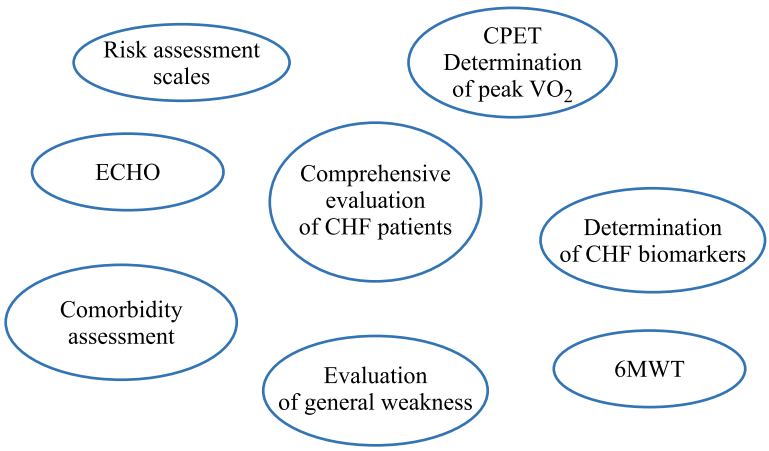

Fig. 2. Comprehensive non-invasive evaluation of the functional status of patients with chronic heart failure resulting from systolic dysfunction before heart transplantation. CHF, chronic heart failure; 6MWT, 6-minute walk test; CPET, cardiopulmonary exercise test; ECHO, echocardiography

CONCLUSION

Today, there are a number of methods for assessing CHF severity. They allow a comprehensive assessment of the patient, determine the survival prognosis, as well as indications for continuation of drug therapy or the need for surgical treatment of the end-stage CHF, which involves resynchronization therapy, implantation of long-term mechanical circulatory support systems and/or heart transplantation. The prognostic risk assessment model for CHF patients is multiparametric, including many variables obtained during clinical and instrumental examination of the patient. However, the relevance of individual prognostic markers may vary depending on the severity of CHF symptoms and the presence of comorbidities.

Fig. 2 schematically shows the main parameters for assessing the prognosis and planning of further tactics, which, in our opinion, are widely available in the practice of medical institutions.

So, a personalized approach to choosing further treatment tactics for CHF patients largely depends on the survival prognosis for a particular patient. Predicting the future of a patient with heart failure is not a perfect science, but a quantitative assessment of risk factors, which is the beginning of choosing a treatment tactic.

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CAPABILITIES OF INTRAVASCULAR IMAGING TECHNIQUES IN THE DIAGNOSIS OF CARDIAC ALLOGRAFT VASCULOPATHY: LITERATURE REVIEW

A.Yu. Kolesnikov, A.A. Arnt, N.A. Kochergin

Research Institute for Complex Issues of Cardiovascular Diseases, Kemerovo, Russian Federation

Cardiac allograft vasculopathy (CAV) is a coronary heart disease (CHD), arising after an orthotopic heart transplant (OHT), and it is one of the leading causes of death in heart recipients. The probability of death is 10%. CAV can manifest as early as 1 year after OHT. Patients do not have pain syndrome that is typical for CHD due to cardiac denervation. The first clinical manifestations may be congestive heart failure, ventricular arrhythmias or even sudden cardiac death. Coronary angiography is the routine technique for CAV detection. However, it is not sensitive enough (about 44%) for CAV detection at an early stage of the disease. Today, intravascular imaging methods (intravascular ultrasound, optical coherence tomography), which allow the evaluation of the morphology of coronary artery lesions, including CAV, have become widespread. This article is devoted to the modern capabilities of intravascular imaging methods in the diagnosis of CAV. CAV is the main cause of myocardial infarction and chronic heart failure in patients after OHT. Intravascular imaging techniques allow early detection of this condition and prevention of unfavorable outcomes in a complex category of heart recipients. Given the advantages of optical coherence tomography (OCT) and disadvantages of intravascular ultrasound (IVUS), OCT appears to be a more informative method of CAV detection.

Keywords: cardiac allograft vasculopathy, orthotopic heart transplantation, intravascular ultrasound, optical coherence tomography.

INTRODUCTION

CAV is a unique form of coronary artery disease that occurs after orthotopic heart transplantation (OHT) [1]. CAV can manifest as early as 1 year after heart transplantation. The median survival of CAV patients is 14.8 years. The prevalence of CAV at 1, 5, and 10 years following cardiac transplantation is estimated to be 8%, 29%, and 47%, respectively. This pathology leads to recipient death in 10% of cases [2, 3].

Coronary angiography remains the gold standard for diagnosing CAV. The sensitivity of angiography is 44% [4]. However, several studies evaluating the histological structure of the vascular wall of the coronary arteries of transplanted heart have shown that 75% of patients had changes characteristic of CAV despite normal coronary angiography findings [5, 6]. Thus, angiography may not detect CAV at an early stage of the disease [4, 7, 8].

Today, intravascular imaging (IVI) techniques such as intravascular ultrasound (IVUS) and optical coherence tomography (OCT) are widely used to evaluate the morphology of coronary artery lesions, including CAV. This article focuses on the current capabilities of IVI in diagnosing CAV.

PATHOGENESIS OF CAV

CAV is a progressive obliterative disease due to intimal proliferation. It encompasses a constellation of vascular changes characterized by intimal fibromuscular hyperplasia (arteriosclerosis), vasculitis, and atherosclerosis. Not only arteries but also veins are affected. This condition results from a complex and incompletely understood interaction between numerous immune and non-immune factors [9, 10].

Graft endothelial cells play a central role in the development of CAV; they are the first cells recognized by the host immune system, effectively becoming antigens [11]. As a result, antibody production begins. Graft endothelial cells not only play a passive role by being recognized by the host immune system, but they can also initiate the inflammatory cascade by enhancing the major histocompatibility complex (MHC) adhesion and leading to a fibroproliferative response [1, 12].

Immune factors

Adhesion of the polymorphic forms of MHC class I and class II discussed above leads to the development of alloimmune responses. Alloreactive host T cells mediated by T helper cells result in the production of cytokines such as interleukin 2, 4, 5 and 6, tumor necrosis factor-alpha and interferon gamma. These factors pro-

Corresponding author: Aleksei Kolesnikov. Address: 6, Sosnovy Bulvar, Kemerovo, 650002, Russian Federation. Phone: (913) 336-58-29. E-mail: inobi05@yandex.ru

mote smooth muscle cell migration into the intimal layer, their proliferation and deposition in the extracellular matrix. Through chemokines, there is an additional migration of T-helper cells and monocytes, which enhance the inflammatory response [10–12].

The humoral component is a crucial factor in allograft injury after OHT. Donor endothelial cells contain antibodies to human leukocyte antigen on their surface. The presence of donor-specific antibodies in the recipient causes endothelial injury through complement activation and antibody-dependent cell-mediated cytotoxicity. Also, antibodies against human leukocyte antigen can stimulate smooth muscle cell proliferation [11–13]. Numerous endogenous molecules derived from the extracellular matrix as well as cell organelles (e.g., mitochondria, cytoplasm and nucleus) can also stimulate the inflammatory process and consequently CAV development through activation of macrophages and dendritic cells [9, 10, 14].

Non-immune factors

Vascular factors, surgical injury to the graft, and infections can cause vascular damage, increase graft immunogenicity, and lead to an alloimmune response. Donor brain death plays a key role in transplant outcome because there is a large release of catecholamines into the blood, development of endocrine disorders or organ hypoperfusion, leading to ischemic graft injury after surgery [9–11]. In the early postoperative period, reactive oxygen species are produced, which damage the microvasculature and also activate endothelial proliferation [12, 13].

Cytomegalovirus infection can mimic the endothelial surface, resulting in cross-reactivity. In addition, infection can directly activate the proliferation of graft endothelial cells and increase oxidative stress, inducing the production of adhesion molecules and promoting endothelial dysfunction by impairing the regulation and production of nitric oxide [13, 14].

IVUS AS A METHOD FOR DIAGNOSING CAV

Currently, IVUS is becoming the new standard for CAV screening. The use of IVUS in heart recipients began in the 1990s [4, 6]. The use of IVUS has led to significant advances in early detection of the disease. Due to its high penetrating power, ultrasound visualizes the lumen and vessel wall in cross section, which contributes to better diagnosis of CAV (Fig. 1) [1]. A prospective study by Torres et al. compared the sensitivity of coronary angiography and IVUS in the diagnosis of CAV in 31 patients with a mean time after OHT of 3.7 years. IVUS detected evidence of CAV in 54.8% of patients, whereas coronary angiography in 32.3%. The study showed that IVUS is a more sensitive diagnostic tool compared to coronary angiography [1, 15].

A study by Mendiz et al. included a total of 114 post-OHT patients who underwent coronary angiography and IVUS. Mean follow-up was 87 ± 61 months. Lesions documented by coronary angiography were found in 24% of the 114 patients, while IVUS revealed CAV in 76.3% [16].

Intimal thickening is most pronounced in the first year after heart transplantation, which is likely a consequence of the increased immune response early after transplantation. In a multicenter study, Kobashigawa et al. demonstrated that an increase in maximum intima-media thickness (IMT) values ≥0.5 mm from baseline was associated with higher mortality, graft loss, and nonfatal cardiovascular events, as well as a higher likelihood of developing CAV within 5 years [4]. In turn, in a study by Potena et al., changes in IMT ≥0.35 mm 5 years after transplantation were significantly correlated with cardiovascular mortality in 131 patients. In addition, severe intimal thickening (mean IMT 0.9 ± 0.3 mm) was

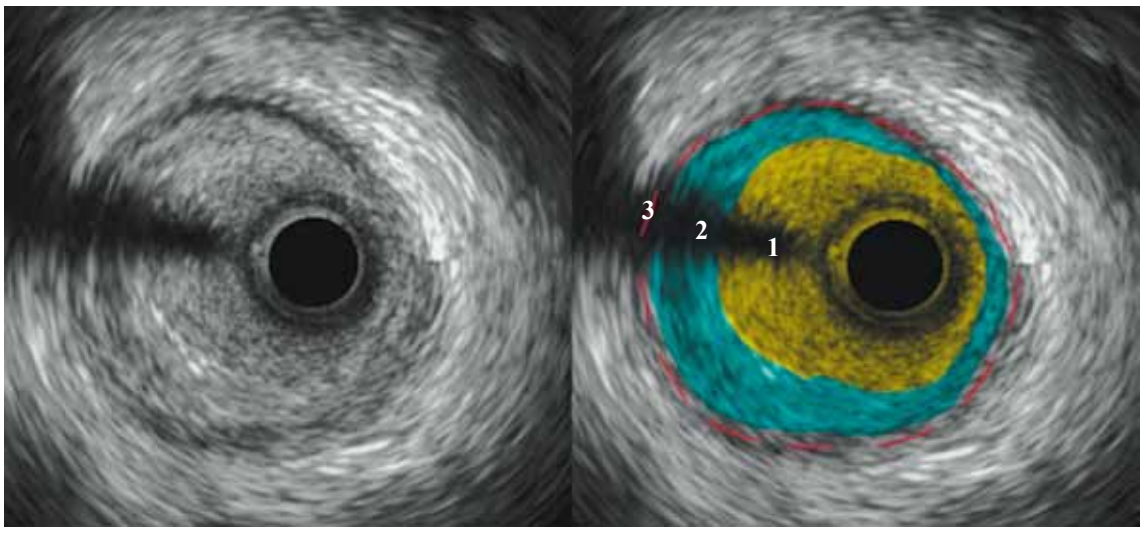

Fig. 1. IVUS in a CAV patient (1, vascular lumen; 2, intimal hyperplasia; 3, media)

associated with a tenfold increase in the risk of major adverse cardiovascular events [1, 17, 18].

IVUS can identify plaque morphology as well as detect CAV progression at an early stage [19–21]. However, despite its effectiveness, IVUS comes with limitations: normal intimal and medial thicknesses are well below the resolution of IVUS (150–200 μm). Early intimal abnormalities, when specific therapies may be potentially more efficacious, are therefore undetectable by IVUS. Also undetectable are pathologically relevant structures such as macrophages, and thin-cap fibroatheromas [6, 22, 23].

OCT AS A DIAGNOSTIC METHOD FOR CAV

OCT is currently considered as an alternative to IVUS for screening in CAV patients [1]. OCT is a technique that uses near-infrared light, which allows for high-resolution imaging. The use of OCT for the diagnosis of CAV is a relatively recent development that has led to a better understanding of the pathogenesis of vasculopathy [14, 19, 20]. OCT can clearly distinguish a wide range of vascular wall components. OCT more accurately represents the intima-media interface, classifying tissue as fibrotic, homogeneous, fibrotic-calcified, with well-defined borders, or with diffuse borders or abundant lipids, allowing detection of intimal hyperplasia ≤150 μm [1, 24, 25]. Fig. 2 shows OCT data in a CAV patient.

To evaluate the efficacy of OCT for CAV screening, the OCTCAV study evaluated 15 patients who had undergone OHT 1 to 4 years previously. All patients underwent coronary angiography followed by OCT. No evidence of CAV was detected by angiography, but OCT revealed neointimal hyperplasia with IMT >1 mm in 8 of the 15 patients. In addition, 7 of the 15 had lipid-rich or calcified atherosclerotic plaques. The researchers concluded that OCT provides high-resolution quantitative imaging of coronary arteries, and it allows detailed assessment of the coronary artery wall and early morphologic changes that occur after heart transplantation [20, 26].

A disadvantage of OCT is its small penetration depth of 1–2 mm [20, 23]. In cases of severe intimal hyperplasia, imaging of the underlying layers is difficult with OCT. Another disadvantage is the need to obtain high-quality images of complete washout of blood cells from coronary vessels [20, 27, 28].

OCT VS IVUS

Compared with IVUS, OCT has 10-fold higher axial resolution (10–15 μm) and provides near histological-level imaging. Structures such as macrophages, plaque fibrous cap thickness, and details of plaque ultrastructure that cannot be imaged by IVUS can clearly be seen by OCT [23, 27, 29].

One of the earliest studies comparing OCT and IVUS in native cadaveric specimens found that intima-media thickness had a higher correlation with histological examination as measured by OCT than IVUS [4]. Early studies have shown the advantages of OCT over IVUS for CAV assessment. Hou et al. assessed the proximal, middle, and distal segments of the left anterior descending artery using OCT and IVUS in 7 long-term heart transplantation survivors. Intimal hyperplasia, defined as an intima >100 μm, was seen in 66.7% of segments by OCT, but only in 14.3% of segments by IVUS. An intimal thickness <150 μm was undetectable on IVUS [4]. OCT is more sensitive in detecting pathologic changes, including vasa vasorum and thin-cap fibroatheromas that is not visible on IVUS [15, 18, 23].

CONCLUSION

Cardiac allograft vasculopathy is the main cause of myocardial infarction and chronic heart failure in patients after OHT. Intravascular imaging allows for early diagnosis of this condition and prevention of unfavorable

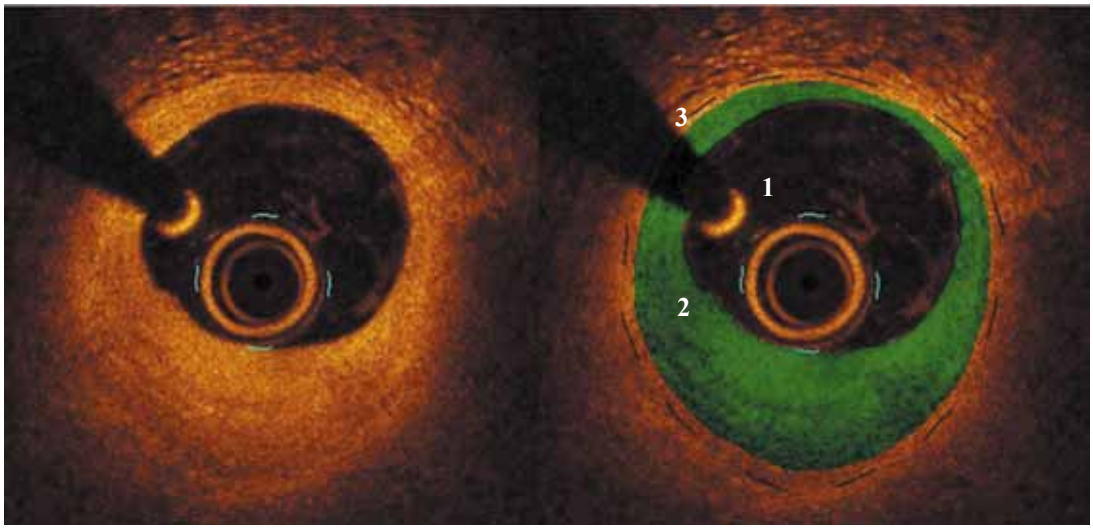

Fig. 2. OCT in a CAV patient (1, vascular lumen; 2, intimal hyperplasia; 3, media)

outcomes in a complex category of heart transplant survivors. Considering the advantages of OCT and disadvantages of IVUS, OCT seems to be a more informative method for diagnosing CAV.

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PREPARATION AND EVALUATION OF A SUSPENSION OF HUMAN CORNEAL ENDOTHELIAL CELLS ISOLATED FROM THE EYES OF CADAVERIC DONORS FOR TRANSPLANTATION IN AN EX VIVO EXPERIMENT

D.S. Ostrovsky[1], S.A. Borzenok[1, 2], B.E. Malyugin[1, 2], O.P. Antonova[1], M.Kh. Khubetsova[1], T.Z. Kerimov[1, 2]

[1] Fyodorov Eye Microsurgery Federal State Institution, Moscow, Russian Federation
[2] Moscow State University of Medicine and Dentistry, Moscow, Russian Federation

Background. According to the World Health Organization, corneal diseases are one of the major causes of blindness globally. Endothelial dystrophy is one of the etiological factors leading to corneal diseases. The corneal endothelium is a monolayer of cells with virtually no mitotic activity. When the density of corneal endothelial cells falls below a critical threshold, the endothelium loses its ability to regulate corneal stromal hydration. This leads to corneal clouding and, consequently, to reduced visual acuity and quality of life of the patient. In this regard, various keratoplasty methods are widely used in clinical practice. Today, it is technically possible to transplant all corneal layers via penetrating keratoplasty, and to transplant the posterior epithelium via layer-by-layer keratoplasty. These surgical approaches are now widely used in everyday practice, but they require the use of scarce material – cadaveric donor corneas, from which grafts for the above-mentioned operations are formed in the conditions of an eye bank. In this regard, protocols for obtaining human corneal endothelial cell (HCEC) culture for subsequent transplantation have been proposed in recent years. However, the use of such approaches in Russia is limited by the law. **The aim** of this study was to experimentally justify the possibility of transplanting uncultured endothelial cells, isolated from cadaveric human corneas. **Materials and methods.** The first stage of the work consisted of obtaining a suspension of endothelial cells from cadaveric donor corneas and studying it; at the second stage, the transplantation effectiveness of the resulting cell suspension was assessed in an *ex vivo* experiment. **Results.** The cell phenotype after transplantation by the proposed method had high viability and preservation. **Conclusions.** The presented results suggest that phenotype and adhesion ability are preserved, and that the cell suspension has a high level of viability under adequate loss of endothelial cells during transplantation in the *ex vivo* experiment.

Keywords: cornea, endothelium, posterior epithelium, transplantation, cells.

INTRODUCTIONS

The cornea is the most important optical medium of the eye, accounting for most of the eye's refractive power. This is due to the unique histological structure of this tissue. The cornea is one of the parts of the fibrous tunic of the eye and it is in direct contact with the environment. For this reason, many different etiological factors can lead to corneal injuries.

According to the World Health Organization, corneal diseases are one of the major causes of blindness globally and are the 4th leading cause of visual disability [1, 2]. In the Russian Federation, about 18% of patients have corneal opacities leading to partial or complete loss of vision. Endothelial dystrophy is one of the causes of corneal opacities.

The posterior epithelium (endothelium) of the human cornea is a monolayer of hexagonal cells located on the inner surface of the cornea. It plays a crucial role in maintaining corneal hydration balance, regulating the inflow of watery moisture to the stroma, thus ensuring corneal transparency. The posterior corneal epithelial cells are practically incapable of mitotic division, and when one of the cells dies, the neighboring cells migrate to the defect site and/or increase in size to close the monolayer defect. A loss of approximately 0.6% of cells per year of life is considered the physiological norm. When the density of CECs falls below a critical threshold of approximately 500 cells per mm^2, the endothelium loses its ability to regulate stromal hydration, which leads to corneal opacity and, consequently, to decreased visual acuity [3].

Keratoplasty is the main treatment for endothelial dystrophies. Today, there has been significant progress in transplantation methods, which is manifested in transition from end-to-end keratoplasty, implying replacement of all corneal layers, but accompanied by a high risk of complications, to direct transplantation of Descemet's

Corresponding author: Dmitriy Ostrovskiy. Address: 59a, Beskudnikovsky Bulvar, Moscow, 127486, Russian Federation. Phone: (499) 488-85-58. E-mail: dmitriy.ostrovskiy@gmail.com

membrane with endothelial cells. This has significantly reduced the risk of complications [4]. However, due to severe shortage of donor material all over the world, there are ongoing activities aimed at obtaining human endothelial cell culture for subsequent transplantation.

In 2018, a group of Japanese scientists led by Prof. S. Kinoshita for the first time successfully transplanted cultured CECs with the help of a rho-associated protein kinase inhibitor in patients diagnosed with bullous keratopathy. A prerequisite was that after the procedure, the patient had to stay in a prone position for 3 hours. This technique showed high clinical efficacy in the long-term follow-up period. Five years after surgery, normal corneal endothelial function was restored in 10 out of 11 operated eyes, endothelial cell density (ECD) of the central part of the cornea was 1257 ± 467 cells/mm^2, visual acuity increased statistically significantly in 10 eyes [5].

In the same year, a group of scientists from Japan and India presented HCEC transplantation results on three patients diagnosed with bullous keratopathy, who did not respond positively to drug therapy. Transplantation of cultured CECs into the anterior chamber was performed using nanocomposite gel to reduce cell migration into the structures of the eye's anterior chamber. A prerequisite was that after the procedure, the patient had to stay in a prone position for 24 hours. Cells from cadaver eyes were cultured for 26 days, then the resulting cell culture was transferred to the gel carrier, where co-culture lasted for one week. The resulting gel carriers were transplanted into 3 patients via a 23G cannula. Endothelial defect in the patients closed after 11 days. The gel carrier was then removed from the patients' anterior chamber. In two out of three cases, a significant increase in visual acuity was achieved at a follow-up period of up to 18 months.

One of the key issues of endothelial cell transplantation is the study of their native phenotype and its preservation in the process of isolation and cultivation. To study the phenotype of endothelial cells, the immunocytochemical method of staining for the following markers, found in almost all scientific reports, is most often used: positive Na^+/K^+-ATPase; ZO-1, Ki67 and negative vimentin and a-SMA. Many researchers take this set of markers as a basic characteristic of HCECs [6].

However, the presented methods have several limitations for implementation in the Russian Federation due to the Federal Law "On Biomedical Cellular Products", which prohibits the direct use of cultured cells in the hospital. The aim of this study was formulated to create an original endothelial cell transplantation technique that would not contradict the above Federal Law.

Aim: experimental validation of the possibility of transplanting uncultured endothelial cells isolated from cadaveric human corneas.

MATERIALS AND METHODS

Stage 1

Obtaining viable and phenotypically intact endothelial cells from cadaveric human corneas

To perform this stage, nine corneas with viable endothelium (but non-transplantable corneas due to the presence of stromal lesions) were obtained from the Eye Tissue Bank. Studies using biomaterials isolated from human cadaveric eyes were conducted in accordance with officially accepted procedures and special authorization under the laws of the Russian Federation. Donor mean age was 53 ± 4 years, the male/female ratio was 5/4, respectively. The average time from death to entry into the experiment was 20 ± 3 hours. According to the viability classification proposed by S.A. Borzenk, the corneas obtained were of grade 1A (non-transplantable corneas).

Isolation of endothelial cell suspension

Endothelial cell suspension was isolated from cadaveric donor corneas according to the following protocol. First, Descemet's membrane with endothelial cells was surgically isolated. Then the Descemet's membrane and endothelial cells were transferred into a tube for enzymatic treatment with chemically stable trypsin TrypLE (Thermo Fisher Scientific, USA), after which the tubes were transferred into a TS-100 thermo-shaker (BioSan SIA, Latvia) using the following device settings: constant heating temperature 37 °C, 800 rpm, 40 minutes. At the end of this procedure, the tubes were centrifuged, the supernatant was removed, and a culture medium of the following composition was added: DMEM/F12; 5% fetal bovine serum; 2 mM L-glutamine; L ascorbic acid; epidermal growth factor (EGF); insulin; Rho-associated protein kinase (ROCK) inhibitor Y-27632; antibiotic and antimycotic solution in a 1 mL volume, after which cell counting was performed on an automated cell counter Luna II (Logos, South Korea).

Determination of viability

The viability of the resulting suspension was determined by fluorescent staining using a commercial kit "Live and Dead" (Abcam plc., UK). This kit represents fluorescent dyes that stain dead cells in red color and live cells in green color. For analysis, 5 mL aliquots were taken from the resulting suspension and mixed with 5X dye solution for subsequent confocal microscopy. Evaluation was performed using a confocal laser scanning biological microscope FluoView FV10i (Olympus Corporation, Japan).

To determine viability and apoptosis, 7AAD and Annexin V dyes were used; for this purpose, 50 μL of suspension was taken and mixed with dyes. The analysis was performed on a CytoFLEX flow cytometer (Beckman Coulter, USA).

Flow cytometry

To confirm the conservation of endothelial cell suspension phenotype, flow cytometry was performed according to the protocol on a CD panel proposed by Kinoshito. Protocol description: 3 tubes of 1×10^5 cells each were formed, isotopic controls were added to 1 tube, CD166 APC 750, CD44 PC7, CD24 PE were added to 2 tubes, CD105 APC, CD26 PC7, CD133 FITC were added to 3 tubes. The analysis was performed on a CytoFLEX flow cytometer (Beckman Coulter, USA).

Stage 2

Assessment of transplantation efficiency for the resulting cell suspension in an ex vivo experiment

For the second stage, cadaveric donor corneas with a viability score of 1A, n = 4. Transplantation of the cell suspension onto pre-prepared cadaveric donor corneas with a standard defect zone was carried out under sterile conditions at the rate of 1,000 cells/mm^2, followed by cultivation for 1 week in complete cell culture medium of the following composition: DMEM/F12; 5% bovine calf serum; 2 mM L-glutamine; L ascorbic acid; EGF; insulin; ROCK Y-27632; antimycotic and antimycotic solution. For transplantation, we used a syringe with a glass nozzle with a 0.2 ml volume and <2 mL nozzle diameter, which allows optimizing microsurgical manipulations with the cell suspension.

The main stages of endothelial cell suspension transplantation included the mechanical removal of native endothelium using a sterile swab, detection of defect site by trypan blue stain, transfer of prepared endothelial cell suspension using a glass nozzle to the cornea at 1,000 cells/mm^2 per 100 μL of complete cell culture medium. Next, the corneas were cultured in 7 mL of complete cell growth medium in a 6-well plate. Cultivation lasted for 7 days.

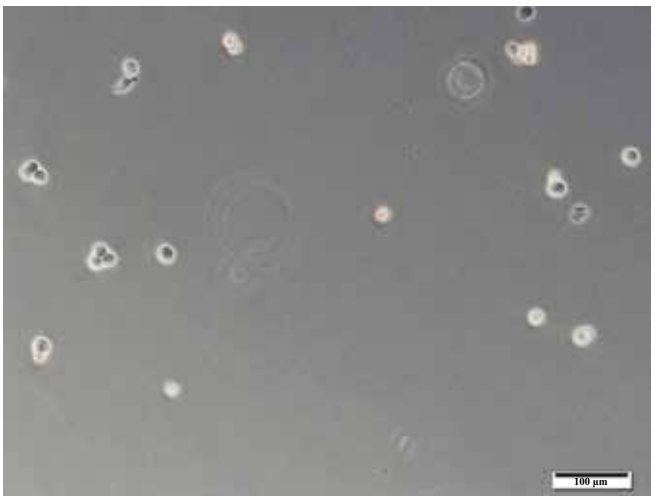

Fig. 1. Endothelial cell suspension. Light phase-contrast microscopy; 100× magnification

Immunohistochemistry

At the end of cultivation, to verify the functional activity of the transplanted posterior corneal epithelial cells, immunohistochemistry (IHC) was performed for the following markers: tight intercellular junction protein (ZO-1), functional proteins Na^+/K^+-ATPase, Lumican, Vimentin and proliferative activity protein Ki67. The corneas were washed once with sterile phosphate-buffered saline (PBS), then fixed in 10% formalin for 24 hours. The protocol of the IHC study included the following main steps: Permobilization with 0.1% Triton X100, blocking of non-specific binding with 0.3% Tween 20 and 1% albumin solution, incubation with primary and secondary antibodies, nuclei were counterstained with Hoechst #33258 nuclear dye. Analysis was performed using a FluoView FV10i confocal laser scanning biological microscope (Olympus Corporation, Japan).

Electron scanning microscopy

Corneal electron microscopy was also performed to determine the quality of closure of the formed defect by transplanted endothelial cells at the end of culturing. The obtained samples were washed once with sterile PBS, then fixed in 10% formalin for a day. The specimens were dehydrated in an ascending battery of acetone, followed by critical point drying, gold sputtering, and analysis on a Jeol 6000 plus electron-scanning microscope (Jeol, Japan).

RESULTS

Stage 1

At this stage, a suspension of endothelial cells was isolated, viability was assessed and the number of cells in apoptosis was determined. It was shown that with the enzymatic method of isolation, the suspension consisted of single cells with single small clusters of 5–10 cells (Fig. 1). The number of viable cells in the resulting suspension was 98.07 ± 1.21% (Fig. 2). The number of cells in a state of apoptosis was 0.1 ± 0.012%, dead cells 1.53 ± 0.61% (Fig. 3).

Flow cytometry showed an expression of more than 98% of CD166, low expression of CD105 and CD133, with no expression of CD24, CD26, and CD44, which is consistent with the results obtained by Kinoshito et al. (Figs. 4, 5).

Stage 2

For transplantation of endothelial cell suspension, it was proposed to use a glass nozzle, which is used in surgery. The main advantages of this nozzle include weak adhesion of cells to the glass walls of the nozzle, internal volume of the nozzle, which allows the cell suspension not to contact with the syringe piston, and small nozzle diameter, which allows to optimize surgical manipulations. Previously, we found that loss of endothelial cells in the glass nozzle is 10% ± 2.5%, and with this method

of transplantation is 40% ± 5.5%. The author's method of transplanting the prepared endothelial cell suspension is presented in Fig. 6.

IHC was performed after 1 week of culturing the cell suspension on the corneas. As a result of the study, expression of the characteristic markers of endothelial cells was detected in the corneal samples: ZO-1 protein (marker of dense intercellular junctions), Na^+/K^+-ATPase (marker of intact pumping function), Ki67 protein (marker of proliferative activity), with the presence of single cells expressing vimentin (protein of connective tissue cell cytoskeleton) and Lumican, which plays an important role in cell migration and proliferation during tissue repair and is also responsible for regulation of the assembly of the collagen matrix in the cornea. The images of endothelial cells obtained during IHC are presented in Figs. 7–9.

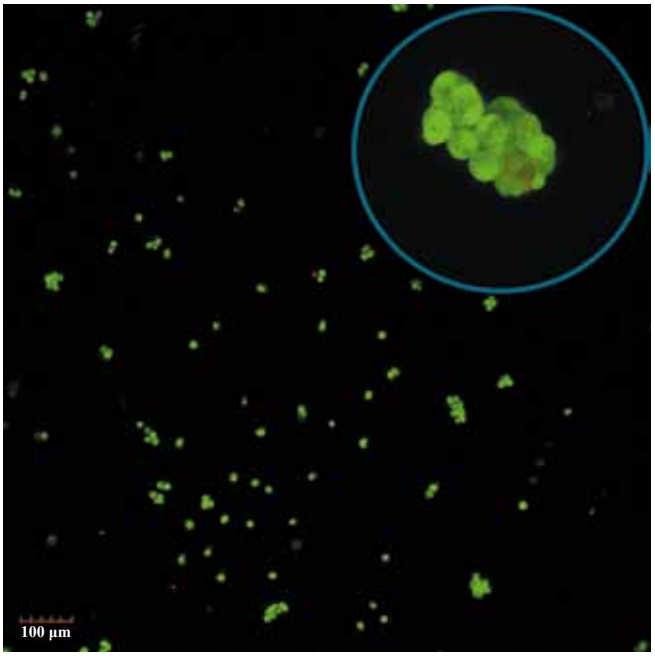

Fig. 2. Determination of endothelial cell suspension viability: live cells are stained in green, dead cells in red. Immunohistochemical staining, confocal laser scanning microscope; 100× magnification

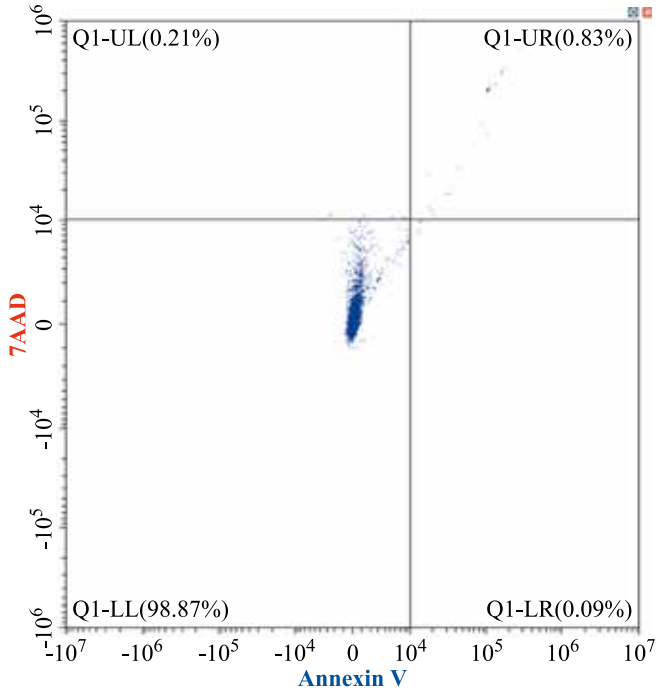

Fig. 3. Determination of viability and apoptosis of endothelial cell suspension. Flow cytometry

Scanning electron microscopy was performed to assess the ability of the resulting cell suspension to adhere to Descemet's membrane and create a monolayer subsequently. The results showed that the endothelial cell suspension formed a monolayer and closed the defect area. The images obtained during scanning electron microscopy are presented in Fig. 10. The red dotted lines indicate cell boundaries.

DISCUSSION

The current Federal Law of the Russian Federation "On Biomedical Cellular Products" regulates the use of cellular products in Russia. According to the adopted provisions, transplantation of in vitro cultured CECs is practically impossible today. For this reason, today it is not possible to use published and successfully proven algorithms for transplantation of human corneal posterior epithelial cells [5, 6]. In this regard, a proprietary endothelial cell transplantation method, which does not contradict the current Law, was used in the present study, since uncultured posterior epithelial cells of cadaveric donor corneas are used for transplantation.

As a result of transplantation of endothelial cell suspension from cadaveric donor corneas, it was found that the cells can successfully adhere to the cornea. The success of endothelial cell transplantation was evaluated by IHC and scanning electron microscopy. The phenotype of the obtained cell cultures was studied by several markers such as Na^+/K^+-ATPase and ZO-1. ZO-1 is a characteristic protein of tight intercellular junctions for epithelial cells. Expression of this tight intercellular junction marker is an indication of preservation of the epithelial phenotype of the studied cells after transplantation by the method presented in this work. The pumping function of the transplanted posterior epithelium was assessed by evaluating the expression levels of Na^+/K^+-ATPase. IHC revealed high levels of Na^+/K^+-ATPase enzyme expression. It was therefore concluded that the pumping function of endothelial cells, which is the key in maintaining corneal homeostasis, is preserved after transplantation according to the specified technique. Also, insignificant levels of expression of the vimentin protein show the absence of epithelial-mesenchymal plasticity of cells. The choice of vimentin is justified by the known role of this protein as a marker of mesenchymal cell transformation. All protein markers used in this study are widely used

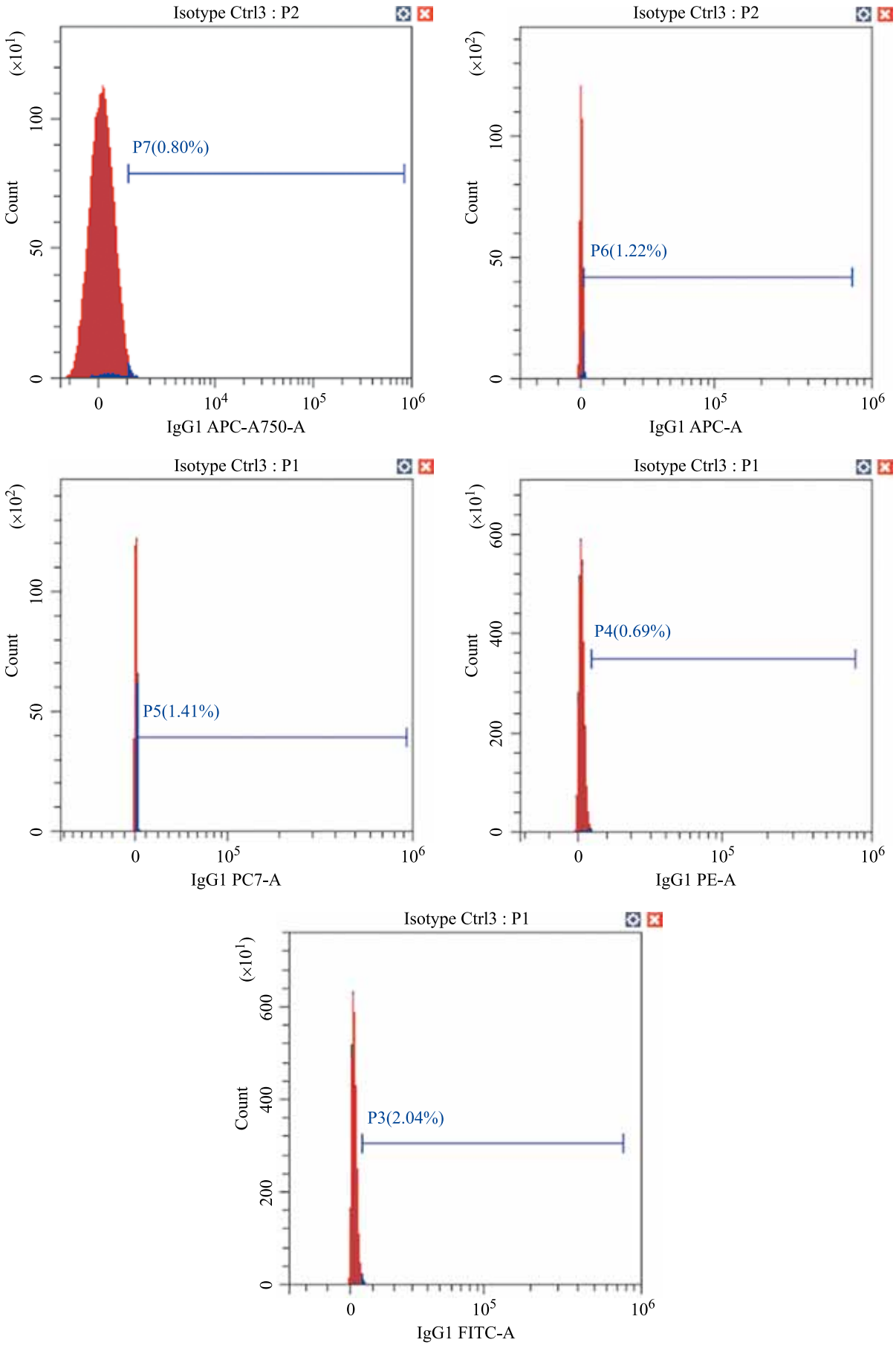

Fig. 4. Isotype control. Flow cytometry

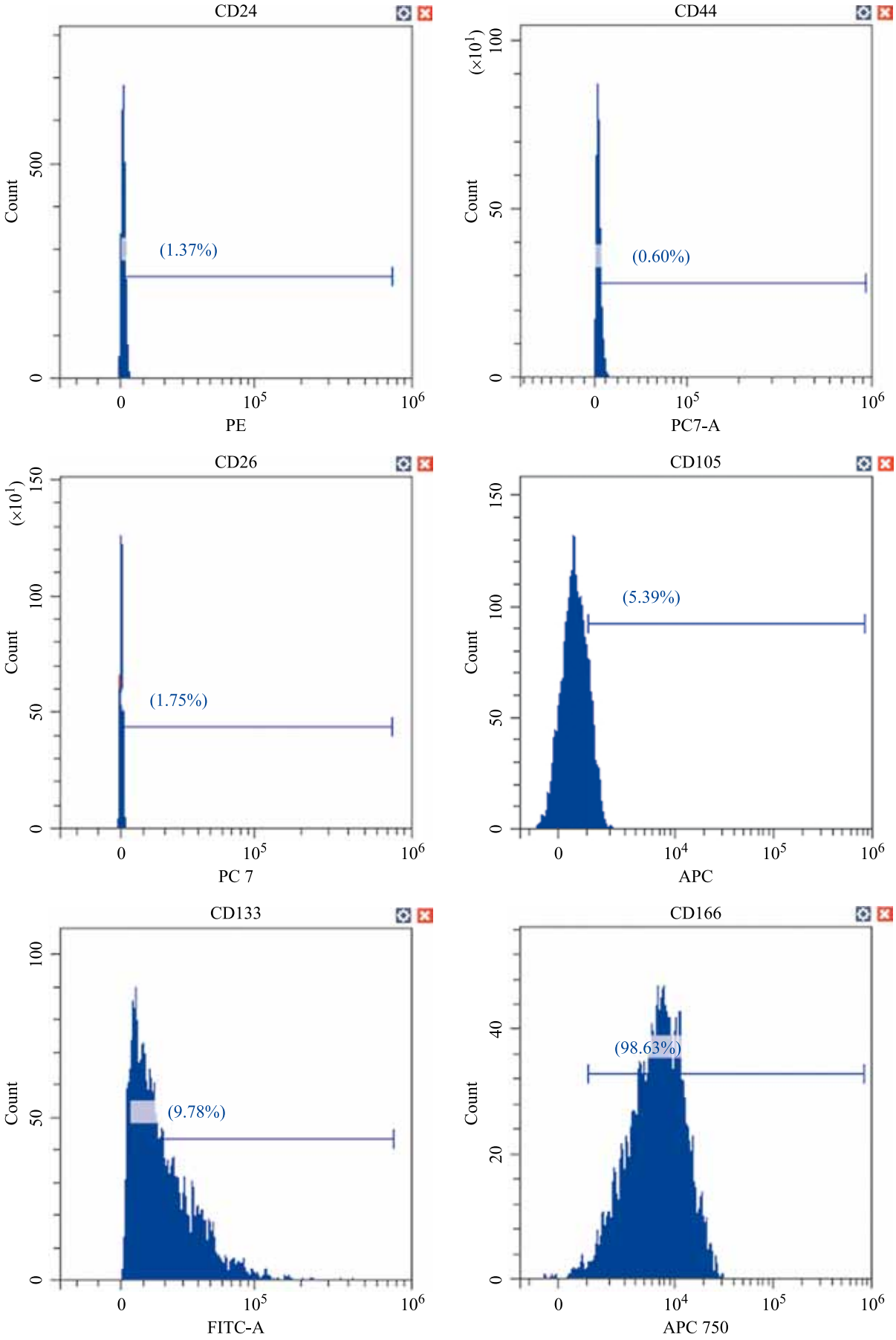

Fig. 5. Flow cytometry of a corneal endothelial cell suspension

in the study of corneal endothelium by various groups of scientists [7, 8].

The dust-like expression of the ZO1 marker was noted, which normally has a honeycomb-like nature. Apparently, this is down to the dissociation of endothelial cell monolayer that occurred when obtaining the suspension. Subsequently, as the monolayer and intercellular junctions are restored, we assume that this marker is fully restored.

The proposed method of transplantation of uncultured posterior epithelial cells using a glass nozzle showed high efficiency and safety for cells. The use of a glass nozzle provided weak adhesion of cells to the glass walls of the nozzle and, thus, ensured low loss of endothelial cells at the direct injection stage. Also, sufficient internal volume of the nozzle allows the cell suspension not to contact with the syringe piston, while the small nozzle diameter allows to optimize surgical manipulations. According to the data obtained, due to the above-mentioned features of the glass nozzle, a <10% cell loss was achieved during transplantation. In known publications, authors also emphasize the need to control transplantation speed to minimize trauma and the most delicate surgery [9]. Such control is possible when using a glass nozzle and performing transplantation according to the described technique.

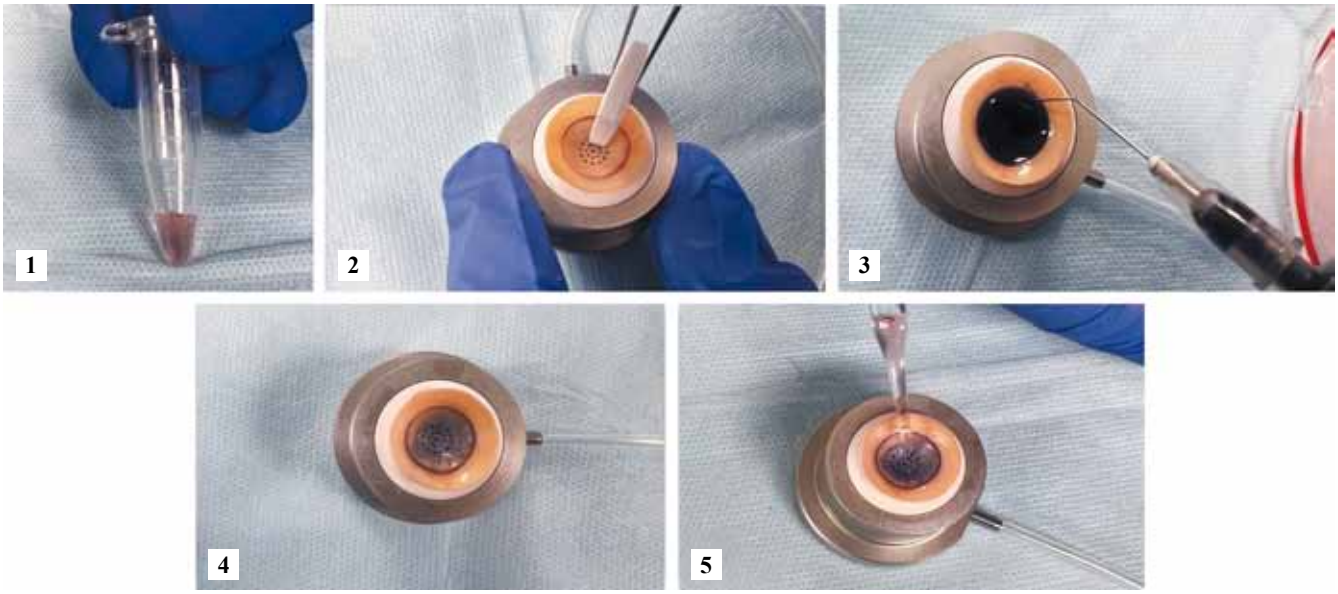

Fig. 6. Method of transplantation of a posterior epithelial endothelial cell suspension, main stages: 1) Preparation of cell suspension; 2) Removal of native endothelium; 3) Staining of defect area with trypan blue, 4) Identification of the defect area, 5) Application of the endothelial cell suspension into the defect area

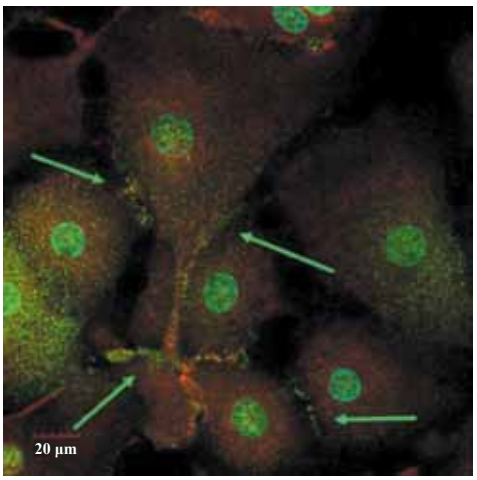

Fig. 7. Immunohistochemical analysis of corneal endothelium. Expression of ZO-1 (green arrows) and Na^+/K^+-ATPase (red stain). Immunohistochemical staining, confocal laser scanning microscope; 600× magnification

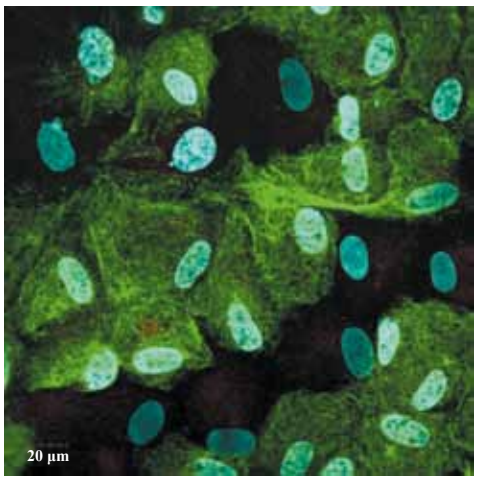

Fig. 8. Immunohistochemical analysis of corneal endothelium. Expression of lumican (green stain) and Na^+/K^+-ATPase (red stain). Immunohistochemical staining, confocal laser scanning microscope; 600× magnification

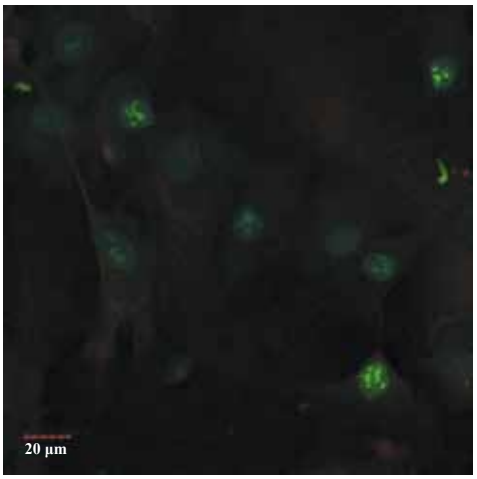

Fig. 9. Immunohistochemical analysis of corneal endothelium. Expression of proliferation marker protein Ki67 (green stain) and Vimentin (red stain). Immunohistochemical staining, confocal laser scanning microscope; 600× magnification

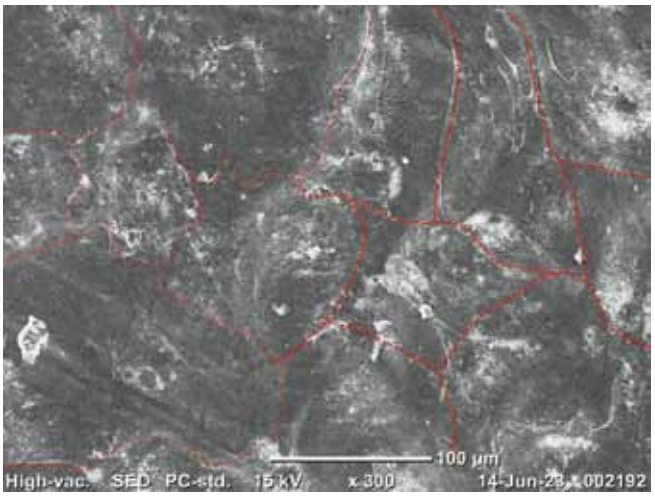

Fig. 10. Endothelial cell layer after culturing on a cadaveric donor cornea. Scanning electron microscopy, 300× magnification

CONCLUSIONS

1. The proposed enzymatic method for obtaining CEC suspension using chemically stable trypsin allows to obtain 98% of viable cells.
2. The resulting endothelial cell suspension expresses 96% CD133, expresses CD105 poorly and does not express CD24, 26, 44. This confirms that the phenotype is preserved after the enzymatic production method.
3. The proposed transplantation method allows minimizing external influence on the endothelial cell suspension and reducing up to 10% cell loss during surgical manipulations.
4. The transplanted suspension of corneal endothelial cells in the experiment showed that these cells can adhere to the Descemet's membrane, forming a monolayer and expressing the characteristic cellular proteins ZO-1, Na^+/K^+-ATPase and Lumican.

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The authors declare no conflict of interest.

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NANOMATERIALS BASED ON CERIUM OXIDE NANOPARTICLES FOR WOUND REGENERATION: A LITERATURE REVIEW

E.V. Silina[1], N.E. Manturova[2], A.G. Erokhina[1], E.A. Shatokhina[1], V.A. Stupin[2]

[1] Sechenov University, Moscow, Russian Federation

[2] Pirogov Russian National Research Medical University, Moscow, Russian Federation

Objective: to analyze data on the synthesis and properties of cerium oxide nanoparticles, as well as the prospects of its application in regenerative medicine for wound healing. **Methodology.** World literature was reviewed using PubMed, SCOPUS, ResearchGate, CyberLeninck, and Elibrary databases, as well as manual searches for authors and reference lists. Key search terms were "cerium oxide" AND nano* AND (healing OR regeneration OR repair) AND wound". The timeline was from the date of publication through August 2023. **Results.** The final analysis included 59 sources containing information on the synthesis and size of nanoparticles (and/or other physicochemical characteristics), methodology and results of in vivo and in vitro studies on the efficacy and/or safety of nanoceria for wound regeneration. It is shown that despite the progressive growth of research interest over the last 15 years, the actual use of nanoceria in practical medicine is still not widespread. This is due to a wide variety of non-standardized synthesis methods and conditions, resulting in the variability of physicochemical parameters of nanoparticles (size, form), thereby affecting the safety and biomedical efficacy of nanoceria. Regeneration mechanisms, including the antioxidant-prooxidant, anti-inflammatory and antimicrobial effects of nanoceria, which contribute to accelerated wound healing, are discussed. The severity of the regenerative effects depends on the method and conditions of synthesis, hence the resulting physicochemical characteristics of the nanoparticles. Therefore, after each batch, newly synthesized nanoceria needs physicochemical and biomedical experimental tests. **Conclusion.** Nanoceria has great potential in tissue engineering for regenerative medicine, particularly for healing of various kinds of wounds. Having developed a technology for standardized synthesis for effective and safe nanoceria (of the right form and size) on a production scale, it can be introduced in medicine, possibly improving the outcomes of treatment of many diseases and pathologies. The authors present conclusions on the results of the study of nanoceria for accelerating qualitative regeneration and the requirements for nanoparticles obtained during synthesis.

Keywords: nanomaterials, nanoparticles, cerium oxide, regeneration, regeneration mechanisms, nanoceria, nanoceria synthesis, size and form, coating, antioxidant effect, antibacterial activity, safety, skin wound healing.

INTRODUCTION

Throughout the history of medicine, issues about wound healing have always attracted robust discussions. This is not surprising because skin wounds still occupy a leading position among all pathologies that require the attention of a doctor. Over such a long period of time, many methods for accelerating the regeneration of damaged skin have been proposed. Natural plant and animal substances, synthetic substances with antimicrobial and regenerative properties, auto- and allogeneic progenitor cells have been used. In the last decade, due to the advent of technologies for the production of nanosized metal salt particles and in connection with some biostimulatory effects inherent in nanoparticles, researchers have shifted their focus to this area. One of the compounds that have demonstrated its ability to stimulate tissue regenerative mechanisms, antioxidant and bacteriostatic activity is cerium dioxide.

Cerium oxide nanoparticles (CeO_2-NPs, nanoceria) have long been studied in biomedical research. According to the international database PubMed, studies on nanoceria have been published for the last 27 years (2281 in number). While the number of such works was in units in the period up to 2005, it was in tens in the period 2005–2009 and in hundreds since 2010. In 2022, 407 publications on nanoceria were registered in the PubMed database. The attractiveness of nanoceria application for regenerative medicine is down to its biosafety, as well as its antimicrobial, angiogenic, and proliferative properties with respect to all cell lines involved in skin regeneration, and antioxidant properties [1–7].

One of the most important characteristics of nanoceria is its redox activity due to its ability to exhibit a trivalent or quadrivalent state depending on the pH of the environment. This makes it unique and extremely reactive. Many researchers have noted this exceptional ability

Corresponding author: Ekaterina Silina. Address: 2, 8, Trubetskaya str., Moscow, 119311, Russian Federation. Phone: (968) 955-97-84. E-mail: silinaekaterina@mail.ru

of nanoceria to switch between oxidation states Ce^{4+} and Ce^{3+}, coexisting on the surface of cerium nanoparticles, depending on the properties and state of the environment, which accounts for its redox-active pro- and antioxidant properties [5, 8–12]. An important feature is the ability of cerium to return the original valence value by adding or removing oxygen atoms with minimal structural reorganizations, which allows the nanoparticle to be used repeatedly in redox reactions [13].

However, despite the progressive increase in research interest, the actual application of nanoceria in practical medicine is still not widespread. There are reasons for this. First, there are several synthesis techniques available now, and production conditions directly affect the final physicochemical parameters of nanoparticles, and hence the (bio)medical result [4]. Secondly, scientific debate on the active properties of nanoceria continues. Regenerative, antibacterial, antioxidant potentials – each of these characteristics has studies confirming or refuting them. Finally, there are various conflicting data on the toxicity of this compound; some works have recorded no death of normal cells up to high concentrations of the substance, while some other reports have shown signs of apoptosis and genomic disorders even at minimal doses. It is likely that the second and third problems are related to the first, since the ambiguous (and sometimes opposite) conclusions by different authors are associated with initially different physical and chemical properties of synthesized nanoceria. This means that only if patterns of influence of the physicochemical characteristics of nanoceria at the molecular, cellular, tissue, organ and, finally, organismal level can be determined that, having developed a technology for standardized synthesis of the most effective and safe nanoceria, with a high degree of probability, its use can be introduced in biology and medicine, possibly improving treatment outcomes for many diseases and conditions.

The aim of this work is to systematize available literature and analyze the data on synthesis and properties of CeO_2-NPs, as well as the prospects for their application in regenerative medicine.

METHODOLOGY

We conducted a review of the world literature using PubMed, SCOPUS, ResearchGate, CyberLeninck, Elibrary, and manual searches by authors and reference lists. The search query used was “cerium oxide” AND nano* AND (healing OR regeneration OR repair) AND wound”. There were no restrictions on publication time (from first publication until August 2023). Inclusion criteria were study material (cerium oxide nanoparticles), skin wound model or other components of regeneration (cell proliferation, migration, scratch test). Types of work: original study, review, meta-analysis, bibliographic analysis, systematic review. Exclusion criteria: micro- or macroceria study.

RESULTS

After analyzing the lists found and removing repetitions, 59 publications were selected for further analysis; 140 sources did not fit the topic of biology/medicine or did not contain information on synthesis and/or size of nanoparticles, or there were no full-text articles (including authors did not send upon request). The literature selection methodology is presented in Fig. 1.

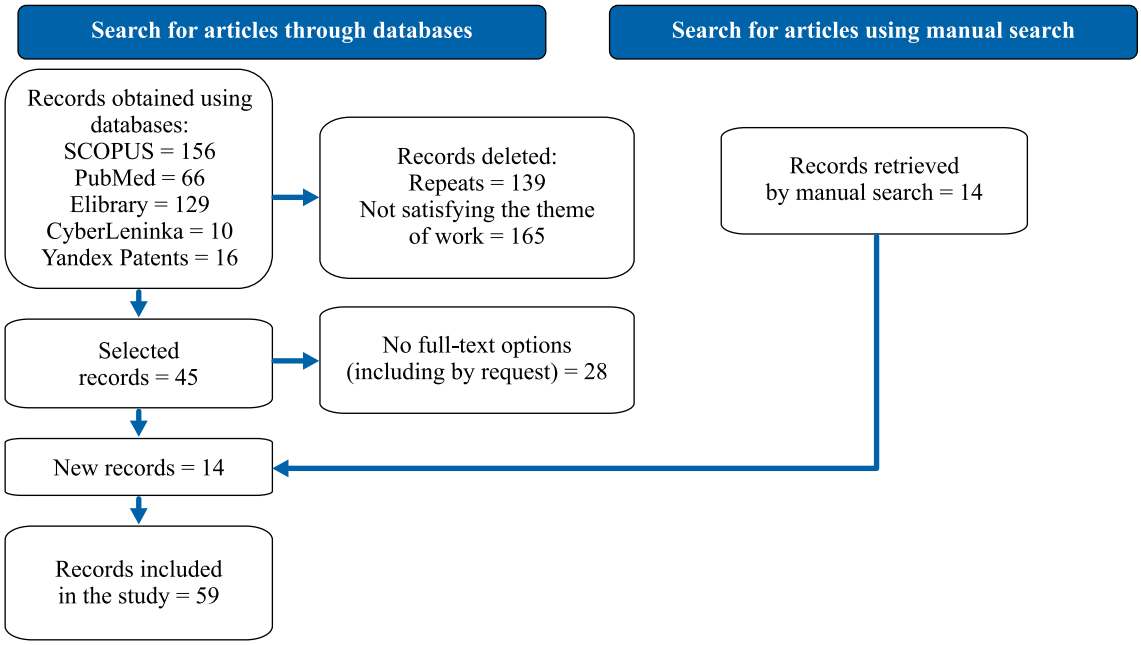

Fig. 1. Summary on the number of articles found by search engines in various databases using the search query “cerium oxide” AND nano* AND (healing OR regeneration OR repair) AND wound” from the date of first publication through August 2023

Out of nearly 200 papers, only a quarter of them contained information on physicochemical (at least 1) properties, method of synthesis (at least briefly), study methodology, and in vitro or in vivo results. However, due to a number of limitations, which are presented here, there is no clinical use of nonoceria yet.

Selection of synthesis technique

To begin with, it is worth dividing CeO_2-NPs synthesis techniques into the following main ones (according to the nature of the synthesis process): physical, chemical and green synthesis. These processes are completely different. Therefore, the results obtained when using the different techniques will be different. In recent years, hybrid (e.g., mechanochemical) synthesis methods combining the first two options have also been emphasized, which is even more associated with the problem of obtaining different results.

The physical method of nanoceria synthesis, which is based on physical processes such as mechanical grinding, melt atomization, and physical vapor deposition, is characterized by a wide range of nanoparticle diameters. Although there are reports on successful synthesis of small particles in a small range (3–5 nm) and possible control of the nanoceria production process [14], however, not all works were able to obtain such results for calibrated particle size [15].

The chemical methods of nanoceria synthesis are based on chemical reactions. These include such types of nanopowder synthesis as chemical vapor deposition, deposition from solutions, high-energy synthesis, reductive processes, and others. Chemical synthesis of nanoceria is characterized by a wide variety of techniques, but among them there are the so-called industrial techniques: low-cost in terms of components and number of reactions [16, 17].

The green synthesis method, based on which metal nanoparticles are prepared immediately with organic coating using natural biological substrates (plants, bacteria, fungi, yeast), will allow obtaining additional properties of the final nanoparticle. For example, there is a synthesis method using Curvularia lunata extract that added antibacterial activity to a nanoparticle that previously did not exhibit such activity; such properties appeared with the use of pectin as a stabilizing and reducing agent [18, 19]. Also, the formed particle obtained bacteriostatic activity (at 2 mM concentration, *E. coli* survival rate 5%, *B. subtilis* 3%). Tannic acid-based synthesis increased the number of oxygen vacancies [20]. In another work, curcumin was used in a composite with nanoceria, additionally with anti-inflammatory effects; the biological efficacy when synthesized in combination with curcumin was higher than directly with cerium alone [13]. In addition, curcumin is a non-invasive indicator of the pH environment, which can theoretically control the progress of wound healing [21].

In addition to the variety of chemical and physical methods of nanoceria synthesis, there are other classifications of nanomaterials production methods: by the aggregate state of starting materials (gas-phase, solid-phase (from solid materials), liquid-phase (from solutions)); by type of nanoparticle formation and assembly technique (condensation from atoms or molecules) or dispersion (deformation), and others. All variables, including degree of temperature and duration of temperature exposure during the synthesis process, can affect the final result.

When choosing a synthesis, it is worth keeping in mind that there is evidence that processing CeO_2-NPs at high temperatures leads not only to loss of antioxidant activity but also to acquisition of pro-oxidant properties [5, 9, 22, 23].

Also, properties of the resulting nanoparticles of the same shape and size differ depending on the precursors. For example, precipitation from Ce(IV) solutions leads to formation of more stoichiometric nanoparticles compared to samples synthesized from Ce(III) salts [24].

Nanoparticle shape and size

The size and shape of the particles determine the redox activity of nanoceria, and the biological activity exhibited [22, 23], so these nanoceria characteristics are fundamental.

Currently, the following are the main synthesized shapes of nanoceria: spherical, cubic, rod-shaped, octahedral, rhombohedral, and spiked. The latter has been reported to be more effective in forming nanobridges that promote faster wound healing compared to the control [15]. Most likely, it was this with the spiky form that promoted regeneration, as the other effects (antioxidant, antimicrobial) were not significant. The biggest influence is the ratio of particle surface area to particle size. For example, smaller spherical and octahedral particles, approaching spherical, show better permeability through the cell membrane compared to larger particles due to penetration into cells through the energy-independent cellular uptake pathway [25]. Nanoparticles in the form of rods provide the best orientation-dependent interaction with cell surfaces; the rods contain increased levels of cerium in the Ce^{3+} valence. However, it was the cubic shape that had the greatest antioxidant effect, which the authors of the study attribute to the open face of the shape and the Ce^{3+}/Ce^{4+} ratio. Moreover, they suggest that Ce^{3+} rods form stable $CePO_4$ compounds and crystal $CePO_4/CeO_2$ complexes, reducing antioxidant abilities with the possibility of toxic effects [25].

Nanoparticle size matters more than anywhere else in nanochemistry. Smaller particles have the highest percentage of potentially active surface area, so agglomeration (clumping of nanoparticles) is undesirable. Moreover, according to researchers, when particle size is less than 10 nm, their toxicity decreases dramatically

due to the sharp increase in oxygen non-stoichiometry of particles and their reductive activity [24].

Nanoparticle coating

Along with unique suitable properties for use in regenerative medicine, nanoceria has several disadvantages that limit its use. The first is aggregation (agglomeration), which leads to loss of activity, and hence useful biological properties, against the background of increased toxicity. Higher $Ce^{3+/4+}$ reactivity is associated with higher toxicity. In addition, the highly reactive nature of nanoceria is associated with non-specificity of interaction, as well as with loss of stability of nanocomposites. Formation of the so-called protein corona (protein adsorption on nanoceria surface) can negatively affect interaction with cells and the nanoceria excretion processes [11, 26].

To overcome these limitations, to improve nanoceria stability, various methods of nanoparticle surface coating are being attempted. In addition, coating a nanoparticle achieves several advantages: stabilizing the shape and size, improving solubility, increasing the half-life of excretion, improving permeability and further stages of pharmacodynamics and pharmacokinetics [27]. Sometimes it increases biocompatibility and acquires additional useful qualities.

Various types of ligands can be used as shells to cover the surface of cerium nanoparticles: different types of polymers (polysaccharides, particularly dextran, polyethylene glycol, polyacrylic acid), carboxylic acids (citrate), polyoxometalates, silanes, peptides, and many others.

The choice of shell depends on initial particle data (particle stability, solubility) and the resulting effects required (potentiation of antioxidant effects, anti-inflammatory or antibacterial additional properties).

Dewberry et al. proposed placing microRNA-146a around cerium oxide nanoparticles. MicroRNA acts on the anti-inflammatory pathway NFkB, thus capturing the three major mechanisms of wound healing (anti-inflammatory, pro-angiogenic, antioxidant,) allowing for qualitative and more efficient acceleration of regeneration. However, the authors note that these nanoparticles can turn into agglomerates that decrease their biological efficacy [28, 29].

CeO_2-NPs containing polyethylenimine and glutaraldehyde interact with superoxide dismutase and catalase, increase their antioxidant potential and protect DNA and proteins from oxidative stress [18].

Dosage form

Nanoparticle delivery form plays a major role in the effectiveness of wound healing. Firstly, the form determines the penetration of nanoparticles into the area of action. Secondly, the rate and duration of release, and thus the efficacy and even toxicity of the substance all depend on the form. Table reviews the main form described in the works so far, their advantages and disadvantages.

Table

Main forms of nanomaterials synthesized to accelerate wound regeneration (healing)

Form	Advantages	Disadvantages / challenges	Source
Gel	• Easy to use on your own • Can mechanically protect the wound surface. • Does not require special skills and frequent changes • With proper choice of materials, it ensures gas exchange • Gel can act as a matrix for proliferation and migration of cells involved in skin regeneration and/or mesenchymal progenitor cells	• It is essential that the material has good biocompatibility and allows gas exchange (e.g., limitation for the use of gelatin). • Uniform release of cerium oxide nanoparticles • The consistency of the medical product should not give a feeling of discomfort, tightness, stickiness	Articles [13, 30–35] Patents [36, 37]
Sols/ solution	A relatively simple dosage form to manufacture	• CNPs are poorly soluble and form conglomerates; it is necessary to stabilize them and increase their permeability with a shell. • Liquid, needs to be applied to the wound frequently • No additional mechanical protection	[38, 39]
Skin films; Plaster/ bandages	• Convenient form • Mechanical protection • Does not require frequent changing, so does not re-injure the regeneration site	• Painless removal of plaster from the wound without damaging the wound • Gradual release of the active ingredient • Gas exchange • Slow degradation of the material, its compatibility	[19, 25, 26]
Injection	Immediately injected (under the skin)	Need for additional equipment, skills	[40]
Lyophilized sponge	Absorbs exudate	If repeated injections are required, there is a risk of introducing infection or damaging healed tissue	[41]

As we can see, the ideal form that would satisfy all needs is yet to be proposed.

Most researchers prefer the gel form, which is due to the ease of use, wider choice of a gel base (alginate, collagen, gelatin, polyvinyl alcohol, etc.) and relatively uncomplicated technology of synthesis and combination with cerium dioxide nanoparticles. Other forms are used less frequently, as this is associated with several difficulties: the technique of introducing the active substance into the carrier, synthesis of the matrix itself with uniform release of nanoparticles, ease of use for the patient, and much more.

We emphasize that all forms of both pharmaceuticals and medical products, including implants, scaffolds, and biosensors, have potential for use in regenerative medicine.

The challenges of developing scaffolds using nanomaterials to accelerate wound regeneration have not yet been sufficiently resolved. Although considering the ever-increasing number of lesions in modern warfare, the problem of treating extensive wounds with large soft tissue defects and depressions on the background of gross cosmetic defects will become among the main problems [6, 42, 43].

Effect of pH on the valence state of cerium

Fig. 2 shows the influence of the environmental condition on the valence state of cerium and hence on its abilities. Considering that the pH of a wound changes during regeneration or progressive microbial contamination, it has been suggested that nanoceria may potentiate the regenerative effect of other drugs against the background of bacteriostatic effects. Such nanoceria potential gave rise to the introduction of the term "smart" drug into medical literature [44].

Studies sometimes use the method of observing the change in color and homogeneity of the solution to determine the readiness of the compound during synthesis [34, 46]. There has been a proposal to use pH in evaluating the progress of wound healing, and curcumin added to the dosage form as a non-invasive indicator [21].

Regeneration mechanism of cerium oxide nanoparticles

Most researchers attribute the mechanism of regeneration specifically to the antioxidant effect [30, 39, 47]. Due to reduction of reactive oxygen species (ROS), which are formed during destruction of phagocytes with the formation of "oxidative burst" or "respiratory burst" [48]. Antioxidant reserves are used to reduce ROS, an inflammatory response develops, and the wound becomes chronic [49]. Effective wound healing occurs at low controlled levels of ROS.

There is also evidence that cell migration and proliferation are accelerated in a medium with CeO_2-NPs, but these effects are achieved in neutral or alkaline media due to the antioxidant properties of cerium [15, 39].

In addition, another mechanism of regeneration and stimulation of cell migration – modulation of expression of redox-sensitive genes – has been described [50]. TGF-β was increased in a medium with CeO_2-NPs, which promotes keratinocyte migration [51]. Also, low levels of ROS promote angiogenesis and re-epithelialization through vascular endothelial growth factor receptor (VEGFR) and epidermal growth factor (EGF) [30]. The increased level of VEGFR2 indicates stimulation of angiogenesis, one of the important elements of regeneration [51].

The regenerative effect of nanoceria is also associated with its antimicrobial activity. Many studies are

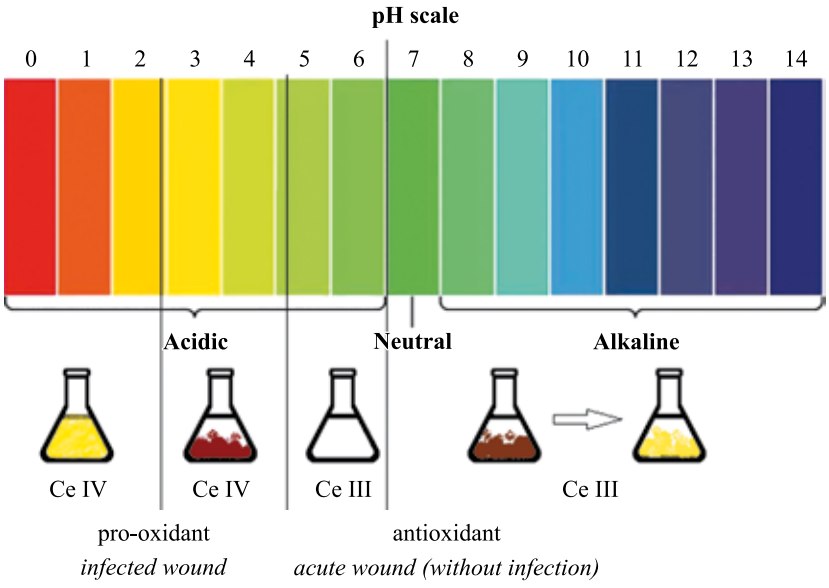

Fig. 2. Relationship between the properties of cerium oxide nanoparticles and pH of the medium [45]

devoted to the antibacterial effect of CeO_2-NPs against gram-positive and gram-negative bacteria [2, 52]. This possibility is down to the following mechanisms. The first one consists in damaging the lipid bilayer of the cell by direct contact between the nanoparticle and the membrane. As a result, the integrity of the bacterial cell is breached, its contents are lysed, and the microorganism dies. By another mechanism, the products of interaction between nanoparticles and intercellular space – ions and reactive oxygen species – have a damaging effect [24]. However, it should be noted that the second way of antibacterial activity is effective only in an acidic environment due to the peculiarities of the valence state of cerium oxide: it is in an acidic environment due to the metabolic products of microorganisms that cerium acquires a 4-valency and pro-oxidant properties with the ability to increase ROS [15].

Antibacterial activity of nanoceria

The question of severity and even presence of antimicrobial activity is still debatable. There are works claiming that nanoparticles of heavy metals or metals with variable valence have a pronounced antimicrobial effect [53–57]. At the same time, there are many works in which the authors did not obtain such an effect [30, 58]. In studies on the antibacterial effect of CeO_2-NPs, the most frequent model for experiments were strains of *Staphylococcus aureus* and *Escherichia coli* [2, 59].

However, the antibacterial effect of nanoparticles has not always been achieved. Several experiments have detected no antibacterial activity [30, 60, 61]. Meanwhile, despite not finding any direct antibacterial effect of CeO_2-NPs, P. Bellio (2018) were able to identify their synergistic effect in combination with antibiotics such as imipenem and cefotaxime [61].

Probably, the presence and severity of antimicrobial activity of nanoceria depends on the method and conditions of synthesis, hence, on the resulting physicochemical characteristics of the nanoparticles. Thus, nanoceria of the right shape and size may prove to be the saving grace that will, if not solve, then alleviate the problem of antibiotic resistance in general and in-hospital infections in particular.

Safety and toxicity of nanoceria

The toxic properties of CeO_2-NPs are also associated with pro-oxidant properties, and as seen in the previous paragraph, they are manifested in acidic environments [24]. Data supporting caspase-dependent cell death are presented in Mittal & Pandey [62]. And also, the results of the study on mice are presented in a review by Rajeshkumar, where reactive oxygen species induced DNA lesion and cell cycle arrest, which caused apoptotic cell death [20]. The same review confirms the dependence of the effect on synthesis conditions and pH of the medium. In one study, a neurotoxic effect was observed in the formation of a complex with serotonin: 5-HT nanoceria, both in the brain and in the intestine (orally with prolonged exposure for more than 3 days) [63]. Meanwhile, in another study, ~10 nm nanoceria prolonged the lifespan and preserved neuronal function while protecting against aβ toxicity and ROS [64]. Considering the multi-enzymatic abilities of cerium depending on the acid-base state of the medium, it can be assumed that cell death is due to the 4-valency of cerium. This behavior of CeO_2-NPs in media with different acidity is the basis for explaining the differential cytotoxicity of the material in relation to tissues with different pH values [65].

In most studies, cytotoxicity increases with increasing levels of nanoceria used [62]. In general, viability of normal cells is maintained up to 10 mM (10^{-2} M); in some studies, the level is even higher compared to the control. In addition, selective toxicity is detected even at low cerium levels for malignant cells that have low pH values; this opens new solutions for targeting malignant tumors in oncology [34]. Some studies have shown that higher levels (up to 250 μg/mL) preserved more than 80% of viable cells [41]. However, there is evidence that bioaccumulation of CeO_2-NPs can cause genotoxic effects, which, however, requires confirmation in long-term studies [66, 67].

CONCLUSION

Thus, it can be concluded that there are great potential prospects for the use of medical products incorporating CeO_2-NPs in the regeneration of wounds of various origins.

Based on analysis of selected publications, we can draw the following initial conclusions about the results of research on nanoceria for early regeneration and the requirements for nanoparticles obtained in the process of synthesis:

1. The most common shape of nanoparticles is the spherical (octahedral) one due to high transcellular permeability or the cubic one. The latter has been drawing attention from researchers for its stability due to its edges and increased restorative capacity resulting from increased number of vacancies.
2. The size should be the smallest possible (<8 nm) with the smallest range. By doing so, a certain degree of stability in the observed effects is achieved. In addition, the size of the nanoparticles in the ash should not differ significantly from that obtained by positron emission tomography (PET) imaging (or other "dry" diameter measurement methods). In such a case, the nanoparticles do not aggregate into agglomerates, which also stabilizes their final in vitro and in vivo performance characteristics.
3. Nanoparticle coating should provide for additional properties that depend on the very nature of the shell. It may be increased stability or solubility. Besides,

the coating may also potentiate the antioxidant or antimicrobial effects of cerium oxide itself.

4. Synthesis method (chemical/physical/"green") should be chosen based on availability of raw materials, technical capabilities of the laboratory, simplicity and ability to repeat experiments so many times with the same result, thus satisfying all the requirements for drugs and/or medical products.
5. The dosage form should be accessible in terms of technology, taking into account mass production, and easy to use for the patient and medical staff without the need for additional training. Such forms are gel and transdermal systems (e.g., patch). However, when synthesizing them, it is necessary to select a material that is biocompatible, stable, able to support gas exchange, absorbs excess exudate, has mechanical protection and ideally antimicrobial-barrier, as well as a structure with matrix properties to facilitate cell migration and proliferation.
6. The pH of the environment (wound) and the dosage form should be taken into account to maximize regeneration.
7. The mechanisms by which CeO_2-NPs participate in regeneration are still debatable at the moment. However, the antioxidant effect of nanoceria (which starts at pH <5) is obviously proven. Antimicrobial effect (its presence and stability) is also discussed: the effect is not shown in all studies in relation to a narrow range of bacteria and is bacteriostatic in nature. There are evidence indicating stimulation of genes responsible for wound regeneration – angiogenic and growth factors.
8. Toxic effects are pro-oxidant in nature and presumably occur only in acidic environments.

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The authors declare no conflict of interest.

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INCIDENCE OF INTRADIALYTIC HYPOTENSION IN HEART TRANSPLANT RECIPIENTS WITH ACUTE KIDNEY INJURY TREATED BY ACETATE-FREE HEMODIAFILTRATION

A.G. Strokov, Ya.L. Poz

Shumakov National Medical Research Center of Transplantology and Artificial Organs, Moscow, Russian Federation

Introduction. Intradialytic hypotension (IDH) is a common complication of renal replacement therapy (RRT) sessions and may be a particularly detrimental factor in heart recipients. **Objective:** to investigate the incidence of IDH in heart recipients with acute kidney injury (AKI). **Patients and Methods:** Two groups of recipients were compared – the study group (SG), n = 313, in which 49 patients required intermittent RRT (IRRT) and in which online hemodiafiltration (OL-HDF) sessions were performed using acetate-free hydrochloric acid-based dialysate fluid; and control group (CG) n = 387, in which 88 patients required IRRT, where standard dialysate with an acetate ion content of 3 mmol/L was used for OL-HDF. **Results.** There was a significantly lower incidence of IDH in the SG compared to the CG: 10.46% *vs* 20.47% ($p < 0.05$). **Conclusions.** In heart recipients for whom IDH can be considered as a significant adverse factor, the use of acetate-free dialysis fluid can significantly reduce the incidence of this complication.

Keywords: heart transplantation, acute kidney injury, renal replacement therapy, hemodialysis, intradialytic hypotension, acetate-free hemodiafiltration.

INTRODUCTION

Heart transplantation (HTx) is the only curative option for patients with end-stage heart failure. The number of HTx is increasing annually; 310 such operations were performed in the Russian Federation in 2022, 212 of them alone at our center, Shumakov National Medical Research Center of Transplantology and Artificial Organs (Shumakov Center) [1]. By the first 10 months of 2023, Shumakov Center had already performed 200 such interventions. One of the most frequent and serious complications after cardiac surgery is postoperative acute kidney injury (AKI), requiring renal replacement therapy (RRT). In a number of cases, this need turns out to be quite prolonged. After patients' condition has been stabilized, intermittent RRT methods replace permanent ones. In such a complex category of patients, ensuring adequate dialysis treatment requires careful attention to all its components, including the composition of the dialysate.

Modern technology for the preparation of bicarbonate dialysate in the proportional mixing system of hemodialysis units involves the use of two-component concentrate, with one component being a sodium bicarbonate solution and the other containing the main electrolytes – sodium, potassium, calcium and magnesium in the form of chlorides. To stabilize the dialysate, prevent precipitation of hardness salts and give it a physiological pH, acid is added to the second component of the concentrate. In the practice of hemodialysis, acetic acid or its substitute, sodium diacetate, is traditionally used, which results in the presence of acetate ion in the dialysate at a concentration of 3–6 mmol/L. The negative effect of acetate ion on the tolerance of dialysis sessions and the incidence of complications, primarily IDH is known. In this regard, elimination of this component from the composition of dialysis fluid can have a beneficial effect on treatment outcomes, especially in patients prone to hemodynamic disorders.

Citric, succinic, and hydrochloric acids are used as alternative acids in dialysate concentrate. Hydrochloric acid is the most appropriate, since replacing acetic acid with hydrochloric acid does not require changes in the routine practice of the hemodialysis center and does not increase the cost of treatment. However, literature data on experience with acetate-free dialysate are limited and fragmentary.

The purpose of this study was to determine the effect of acetate ion replacement in a dialysate composition on IDH incidence in heart transplant survivors with AKI.

PATIENTS AND RESEARCH METHODS

The presented study retrospectively analyzed data from 313 recipients who underwent HTx between January 1, 2022 and June 1, 2023 (study group, SG) and a comparable cohort of 387 recipients who underwent

Corresponding author: Alexander Strokov. Address: 1, Shchukinskaya str., Moscow, 123182, Russian Federation. Phone: (499) 196-88-95. E-mail: medick@bk.ru

HTx between January 1, 2016 and June 1, 2018 (control group, CG), when the switch to hydrochloric acid-based dialysate concentrate was made.

SG was represented by 313 heart transplant recipients, CG by 387 recipients; male patients predominated in both groups; the age composition of the groups did not differ. Among recipients in both groups, patients with dilated cardiomyopathy (DCM) predominated; the number of retransplantations was higher in SG. The proportion of patients with urgent indications for transplantation was also higher in SG, 35.5% UNOS 1B, compared to 12.4% in CG, and the number of patients with UNOS score 2 – 34.0% in SG compared to 57.4% in CG. The baseline characteristics of patients in the two groups are summarized in Table 1.

Intermittent renal replacement therapy (IRRT) was used after the recipients' condition was stabilized on continuous renal replacement therapy (CRRT). Online hemodiafiltration (OL-HDF) sessions in postdilution mode with dialysate/substitute of the following composition was used as IRRT: Na^+, 132–142; K^+, 2.0–4.0; Ca^{++}, 1.5–1.75; Mg^{++}, 0.5; Bic, 30–36; CH_3COO^-, 0–3.0 (mmol/L). Electrolyte composition and bicarbonate concentration were selected individually depending on the electrolyte composition of the patient's plasma and acid-base state parameters. Anticoagulation was performed in the form of dosed administration of unfractionated heparin under the control of activated clotting time. High-flow double-lumen central venous catheters were used as vascular access.

The IDH recorded in treatment session reports was evaluated as a decrease in systolic blood pressure by more than 20 mmHg with the development of clinical symptoms of hypotension and the need for intervention by medical personnel, including increased doses of cardiotonic drugs.

The obtained results were processed using statistical package Biostat; the t-test was used to establish the significance of differences, taking into account the Bonferroni correction. Differences were considered significant at $p < 0.05$ (EpiInfo 5.0, statistical package recommended by WHO).

RESULTS

The need for RRT in the two groups was not significantly different, 33.5% in SG and 34% in CG (Table 2). At the same time, the duration of CRRT, which was performed in the intensive care unit (ICU) immediately after HTx, was significantly longer in SG, 9.85 ± 0.73 days versus 4.57 ± 0.38 in CG ($p < 0.001$). Apparently, longer CRRT was associated with a significantly lower need for IRRT in SG, 15.7% versus 22.7% in CG. IRRT in SG was longer, 20.56 ± 4.9 days versus 13.14 ± 2.03 days in CG, but this difference did not reach statistical significance ($p = 0.163$). A statistically non-significant trend for longer duration of IRRT in SG was observed both when analyzing IRRT duration in ICU, 15.61 ± 8.42 days in SG versus 8.42 ± 1.16 days in CG ($p = 0.122$), and for IRRT duration in the ward, 25.34 ± 8.62 versus 19.70 ± 3.29 ($p = 0.54$).

The main characteristics of IRRT sessions are summarized in.

The number of sessions per week, session duration, ultrafiltration volume (excluding injected fluids), replacement volume (convection volume), and session efficiency according to the Kt/V coefficient did not differ significantly between the two groups. The significance of the differences in the average duration of OL-HDF session and convection volume is determined by many

Table 1

Main characteristics of recipients in the two groups

Indicators	Control group	Study group
Follow-up period	01.01.2016–01.06.2018	01.01.2022–01.06.2023
N (men/women)	387 (341/46)	313 (277/36)
Mean age (m/w)	49.2 (78–13) / 41.7 (70–11)	47.0 (69–12) / 41.1 (62–11)
Pre-HT diagnosis		
DCM	221	195
ICM	154	104
Graft dysfunction	6	11
LVA	4	1
HCM	–	2
RCM	1	–
ACM	1	1
UNOS distribution		
1a	30.2%	30.5%
1b	12.4%	35.5%
2	57.4%	34.0%

Note. DCM, Dilated cardiomyopathy; ICM, Ischemic cardiomyopathy; LVA, Left ventricular aneurysm; HCM, Hypertrophic cardiomyopathy; RCM, Restrictive cardiomyopathy; ACM, Arrhythmogenic cardiomyopathy.

observations. The frequency of IDH episodes was significantly and statistically significantly lower in SG compared to CG, 10.46% vs. 20.47% ($p < 0.05$).

DISCUSSION

The main finding in this study was a significant reduction in IDH incidence during acetate-free OL-HDF sessions in heart recipients with AKI compared with OL-HDF using standard bicarbonate dialysate containing 3 mmol/L acetate ion.

IDH is one of the most frequent complications occurring during hemodialysis sessions [2]. This complication not only reduces treatment effectiveness and prevents adequate ultrafiltration, but also impairs coronary blood flow [3, 4], which can be a particularly unfavorable factor in heart recipients in need of IRRT. One of the mechanisms contributing to the development of IDH may be the influence of acetate ion, which has cardiosuppressive, vasodilatory, and proinflammatory effects [5]. It has been shown that plasma acetate ion levels when using standard bicarbonate dialysate can exceed physiological values by dozens of times, accordingly, its elimination can have a favorable effect on tolerance to IRRT sessions [6].

Most publications concerning the use of acetate-free hemodialysis techniques are devoted to acetate-free biofiltration [7], but this technique requires the use of sterile sodium bicarbonate solution, and the convection volume obtained during the therapy session does not reach the current efficiency criteria. Studies of the effect of acetate-free dialysate on IDH incidence are limited to small groups of patients with end-stage renal failure undergoing program treatment [8, 9]. A Russian study by a group led by T.V. Mukhoedova reported a significant 3.8-fold decrease in the incidence of complications, including IDH, when performing sustained low-efficiency dialysis (SLED) in patients after cardiac surgery [10]. Our study, which included a larger number of heart transplant survivors, revealed a similar relationship. In our study, high-efficiency OL-HDF was performed, duration of sessions was somewhat shorter, and ultrafiltration volume was significantly greater than in the above-mentioned study. It should also be noted that the follow-up period of patients in our study was significantly longer, and recipients with prolonged need for IRRT in some cases were treated as outpatients. Nevertheless, analysis of the entire data set showed a very significant reduction in IDH incidence.

It is worth mentioning that hydrochloric acid used to produce acetate-free concentrate is more aggressive than acetic acid. This should be taken into account both when preparing the concentrate and when using it. In our practice, the steel activator blades in the concentrate mixer were destroyed and the concentrate centralized pump failed (Fig.).

Table 2

RRT need and duration in the two groups

Parameters	Control group		Study group
Needed CRRT	34% (132)		33.5% (105)
CRRT duration (days on avg.)	4.57 (1–19) ± 0.38	$p < 0.001$	9.85 (2–37) ± 0.73
Needed IRRT	22.7% (88)	$p < 0.05$	15.7% (49)
IRRT duration (days on avg.)	13.14 ± 2.03 (1–112), n = 129	NS $p = 0.163$	20.56 ± 4.9 (1–267), n = 63
IRRT duration in ICU (days)	8.42 ± 1.16 (1–67), n = 68	NS $p = 0.122$	15.61 ± 4.46 (1–102), n = 31
IRRT duration in the hemodialysis unit	19.7 ± 3.29 (3–112), n = 61	NS $p = 0.54$	25.34 ± 8.62 (1–267), n = 32

Note. CRRT, Continuous renal replacement therapy; IRRT, Intermittent renal replacement therapy.

Table 3

Main characteristics of OL-HDF sessions in the two groups

Parameters of IRRT sessions (OL-HDF)	Control group	Study group	P
Average number of RRT sessions per week	5.2 ± 0.06	5.06 ± 0.07	NS $p = 0.13$
Average duration of IRRT session (minutes)	300.7 ± 1.35	295.5 ± 1.57	$p = 0.012$
Mean UF volume (ml)	2373 ± 23	2311 ± 24	NS $p = 0.09$
Average replacement volume (l)	15.44 ± 0.08	15.81 ± 0.10	$p = 0.04$
Average Kt/V	1.49 ± 0.05	1.54 ± 0.06	NS $p = 0.42$
Frequency of hypotensive episodes (%)	20.47	10.46	$p<0.05$

Note. UF, ultrafiltration; Kt/V, efficacy of an IRRT session determined by ionic dialysance.

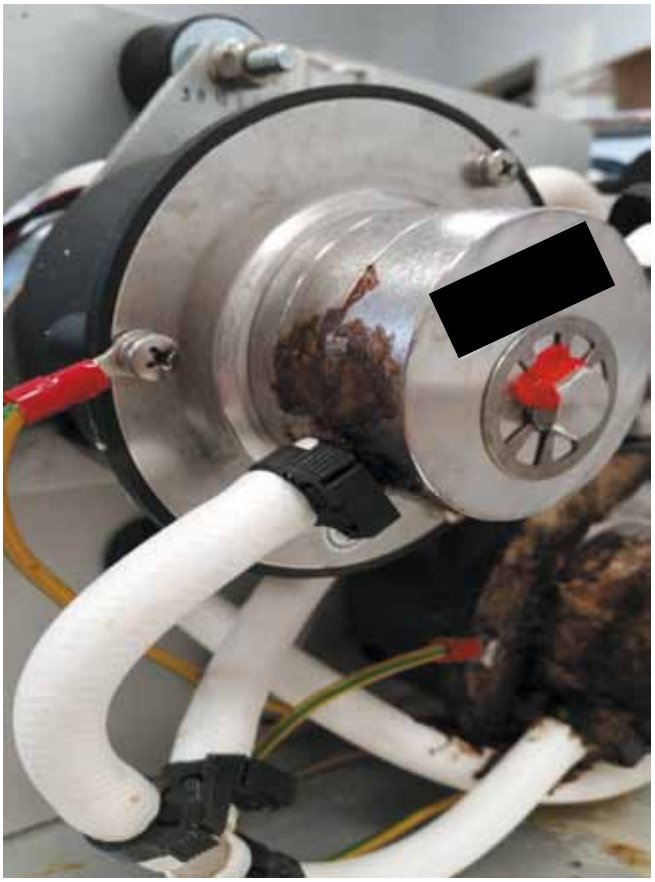

Fig. Corrosion of the central concentrate pump by hydrochloric acid

CONCLUSION

Despite its retrospective nature with uncontrolled collection of clinical data, the large size of the study, which included about 137 heart recipients and about 1700 OL-HDF sessions, allows for the following conclusion: when using an acetate-free hydrochloric acid-based dialysate fluid, treatment of OL-HDF heart transplant recipients is complicated by IDH half as often as when using standard bicarbonate dialysate containing 3 mmol/L acetate ion.

The authors declare no conflict of interest.

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UNCONTROLLED ORGAN DONATION AFTER OUT-OF-HOSPITAL CARDIAC ARREST. LITERATURE REVIEW

M.G. Minina[1], P.A. Drozdov[1], V.M. Sevostyanov[1], E.A. Tenchurina[1], A.A. Nevredimov[1], P.A. Davydov[2], O.V. Shatskova[2]

[1] Botkin Hospital, Moscow, Russian Federation
[2] Puchkov Ambulance and Emergency Medical Care Station, Moscow, Russian Federation

Organ transplantation is the best therapy for terminal and irreversible organ failure. The global development of organ transplantation as a type of medical care is inextricably linked to the establishment of neurological criteria for declaring human death (brain death). In the early evolutionary period of transplantation, organs were used, mainly kidneys, obtained from donors whose death was ascertained in accordance with the generally accepted criteria of cessation of blood circulation and respiration. As this type of organ donation developed, numerous terms were used in the world literature to designate it, such as 'asystolic donors', 'non-heart beating donors', 'donors after cardiac death', etc. In Russia, there is an established practice of dealing with donors after cardiac death (DCD), but the active development of Russian transplantology in the last 20 years is primarily associated with brain-dead organ donation. However, countries with the most active and advanced organ donation practices have in recent years been successfully dealing with donors who have suffered sudden out-of-hospital cardiac arrest (OHCA). Previously, this type of donation was considered inaccessible due to the unacceptable warm ischemia time and consequently severe damage to donor organs. Due to the development of new technologies in emergency medical care, it became possible to transport a patient with clinical death that occurred in an out-of-hospital setting, to the hospital, while providing cardiopulmonary resuscitation by means of automatic chest compression and artificial ventilation. The article presents historical aspects of donation after cardiac death, and the most actualized definitions and practices of dealing with such donors.

Keywords: donors with out-of-hospital cardiac arrest, organ preservation methods, perfusion devices.

BACKGROUND

The first attempts at human-to-human organ transplantation were made in the 1930s in the Soviet Union. Between 1933 and 1939, Yuri Voronoy performed six kidney transplants from deceased donors [1–3]. French surgeon R. Küss developed a heterotopic technique for kidney transplantation into the iliac vessels with ureteroneocystostomy, and in 1951–1952 he performed 8 kidney transplants using his technique [4]. All early attempts at organ (kidney) transplant were associated organ procurement from deceased persons after death had been declared in accordance with the only cardiopulmonary criteria for that historical period, i.e., cessation of blood circulation and breathing [3]. Transplant outcomes were unsatisfactory due to irreversible ischemic injury to the kidneys and the inability to suppress the recipient's native immune system to prevent graft rejection. There was widespread introduction of transplant programs after the discovery of immunosuppressive therapy (azathioprine) in 1960 [5], which, in combination with steroid drugs, was used in the treatment of recipients [3]. During the same period, a new concept for ascertaining human death based on neurological criteria (brain death) emerged. It was after this that the medical world began to talk about the so-called "dualism" of death determination, when along with cardiopulmonary criteria, neurological signs (criteria) of brain death gained legitimacy. Clearly, the state of brain death occurred with incurable cerebral edema in patients on a ventilator in the intensive care unit (ICU). In the 1960s, organs from brain-dead patients began to be considered as possible targets for transplantation. In 1963, the world's first kidney transplant from a brain-dead donor was performed in Brussels [6]. As the practice of dealing with brain-dead donors spread, the frequency of using DCD progressively decreased [3], and to date, brain-dead organ donation is considered the gold standard for deceased human organ donation [7].

CLASSIFICATION OF ORGAN DONORS WITH IRREVERSIBLE CARDIAC ARREST

The term "non-heart beating donation" was adopted in 1995 at the first international workshop on non-heart-beating donors in Maastricht (The Netherlands), where the first Maastricht classification of non-heart-beating

Corresponding author: Elmira Tenchurina. Address: 5, Vtoroy Botkinskiy Proyezd, Moscow, 125284, Russian Federation. Phone: (967) 113-87-64. E-mail: arimle@inbox.ru

donors was formed and presented[1] [8]. The Maastricht classification presents four categories and two types of non-heart-beating donors – uncontrolled and controlled (Table 1).

"Controllability" is determined by the conditions and localization of the onset of cardiac arrest (CA). The uncontrolled type includes those cases of donation where CA occurs suddenly (acutely), and death occurs either upon arrival at the hospital or after unsuccessful resuscitation measures. Uncontrolled organ donation is always accompanied by a limited time interval for possible work with the donor, determined by the total warm ischemia time, taking into account the time taken by the organ donation team to arrive at the donor. Controlled organ donation is performed in conditions when CA is "expected" and donor service specialists are informed about the presence of a possible donor and are ready to start dealing with him, such as in the situation of CA after withdrawal of intensive care (withdrawal of treatment) or CA in donors with confirmed brain death [9–12].

The increasing number of uncontrolled non-heart-beating donors in Europe, as well as the development of organ perfusion technologies, has led to the need to revise the 1995 Maastricht classification and introduce new donor subgroups depending on the location of circulatory arrest onset (out-of-hospital and in-hospital) and the presence or absence of witnesses to the circulatory arrest event. In 2013, the Maastricht classification was modified at the 6th International Conference on Organ Donation after Circulatory Death held in Paris (Table 2) [13].

According to the updated Maastricht classification (Paris, 2013), OHCA donors are categorized as uncontrolled, 1A (sudden OHCA without attempts at cardiopulmonary resuscitation), and 2A (sudden OHCA with unsuccessful cardiopulmonary resuscitation).

Previously, organ donation from OHCA donors was considered unacceptable due to long warm ischemia time and, consequently, the resulting severe injury to the organs. However, the emergence and widespread introduction of automated mechanical cardiopulmonary resuscitation (CPR) devices for performing external cardiac massage, as well as the introduction of extracorporeal oxygenation protocols and methods of ex-vivo perfusion of donor organs, made it possible to reduce the negative impact of warm ischemia, and it became possible to handle this category of donors [14].

Uncontrolled non-heart-beating donation (NHBD) programs began in the 1980s in Spain and the Netherlands, and later in France in 2006. NHBD in Spain was, until recent years, almost entirely was composed of uncontrolled donors. A total of 1,430 uncontrolled NHBD donors were registered in Spain between 2001 and 2016, and their number progressively increased from 17 in 2001 to 138 in 2012. Since 2012, there has been an increase in the number of controlled donors and in 2015, for the first time, their annual number exceeded that of uncontrolled donors, 210 vs 104 [17].

According to the European Committee on Organ Transplantation of the Council of Europe [15], of the 538 NHBD donors registered in Europe in 2008, 137 (25.5%) were uncontrolled donors (Maastricht I and II) and 401 (74.5%) were controlled donors (Maastricht III).

Table 1

Non-heart-beating donors – Maastricht classification (1995) [8]

Category	Description	Donor type
I	Dead on arrival at hospital	Uncontrolled
II	Death with unsuccessful resuscitation	Uncontrolled
III	Awaiting CA	Controlled
IV	CA while brain dead	Controlled

Note: CA, cardiac arrest.

Table 2

Maastricht classification (Paris, 2013) [13]

Category I Uncontrolled	Found dead 1A. Out-of-hospital 1B. In-hospital	Sudden unexpected CA without any attempt of resuscitation by a life-medical team
Category II Uncontrolled	Witnessed CA 2A. Out-of-hospital 2B. In-hospital	Sudden unexpected irreversible CA with unsuccessful resuscitation
Category III (Controlled)	Withdrawal of life-sustaining therapy	Planned withdrawal of life-sustaining therapy*; expected CA
Category IV (Controlled/Uncontrolled)	CA while life-brain dead[2]	Sudden CA after brain death diagnosis during donor life-management but prior to organ retrieval

***** This category mainly refers to the decision to withdraw life-sustaining therapies.

[1] Hereafter in the text, the term "non-heart-beating donors" will be used to refer to donors with cardiac arrest.

[2] This refers to the maintenance of vital functions in a person diagnosed with brain death.

Most uncontrolled NHBD cases were reported in Spain and France [16].

In a retrospective British analysis devoted to the study of outcomes of patients admitted with OHCA, it was shown that over an 11-year period from 2004 to 2014, against the background of higher number of patients with OHCA, there was a substantial increase in uncontrolled donors among deceased patients – from 3.1% to 10.1%, and by now donors with OHCA account for up to 25.0% of the total pool of effective donors in the UK [18].

Most publications on uncontrolled donors do not include cases of unwitnessed OHCA (up to 45.0% of all cases)[19], indicating that there is still significant potential for this type of donation [9].

INCLUSION CRITERIA FOR OHCA DONORS

The most considered criteria are donor age, presence/absence of a witness to the CA, no-flow time (from the time of circulatory arrest to the start of CPR) and low-flow time (from the start of CPR to cannulation and start of organ perfusion). Circulatory arrest resulting from traumatic injury with signs of active bleeding can be considered as a possible obstacle in dealing with uncontrolled donors taking into account heparinization necessary to ensure normothermic perfusion; however, there are reports indicating the possibility of perfusion with the lowest possible doses of heparin and correction of anemia and hematocrit by adding donor red cell mass to the perfusion circuit [9]. Contraindications to organ donation in uncontrolled donors are standard for all types of organ donation – malignant tumors, blood-borne infections, and chronic organ failure (Table 3).

In 2016, B. Domínguez-Gil et al. analyzed the practice of uncontrolled NHBD among European countries – Spain, France, the Netherlands, etc. The results showed the existing differences in donor selection criteria and legal regulation [16]. A retrospective study of the nationwide Out-of-Hospital Spanish Cardiac Arrest Registry (OHSCAR) analyzed data on deceased OHCA patients in Spain for 13 months (October 1, 2013 to October 31, 2014). Inclusion criteria for donation were age 16–60 years, no-flow time <15 minutes, and no return of spontaneous circulation. Of the 3,544 OHCA patients, only 181 (5.1%) met these inclusion criteria and could potentially be considered for donation. An additional group of 154 patients met inclusion criteria such as age and witnessed circulatory arrest, but the no-flow time was not specified. The actual number of OHCA patients who became donors was 141 (4.0%) [20].

Reed and Lua retrospectively studied all OHCA patients in Lothian, Scotland between August 1, 2008 and September 30, 2009 to identify patients who were potential donors with OHCA [21]. Inclusion criteria were age 16–60 years, witnessed circulatory arrest, ambulance arrival within 15 minutes or less, patient death in the emergency department after unsuccessful resuscitation, time from circulatory arrest to certification of death <120 minutes, patient being on the donor register, and patients presenting to the emergency department between 9:00 and 17:00 on weekdays. Of the 564 OHCA patients, 351 had witnessed CA, of which 224 had an ambulance crew arriving on site within a time interval ≤15 minutes, of which 93 patients were admitted to the emergency unit of the hospital on weekdays during scheduled working hours, of which 63 died, of which only 16 were aged between 16 years and 60 years, of which 15 died within 120 minutes of CA, of which only 9 had donor-eligible medical conditions. The present study demonstrates the importance of an organizational algorithm for this type of donation, because the ability to deal with these donors only during working daytime hours significantly limits the number of potential donors. In addition, it is important to periodically review the criteria of donor medical eligibility for possible expansion, taking into account the experience of countries that have been successful in dealing with this type of donor.

Table 3

Inclusion and exclusion criteria for OHCA donors [9]

	Criteria
Inclusion	Lower age limit – 18 years (varies by country)
	Upper age limit – 55–60 years (varies by country)
	Witnessed CA
	No flow time <30 minutes
	Transport time to hospital is <90 minutes from CPR start time
	Registered as an organ donor (where applicable)
Exclusion	Trauma, active bleeding
	Cancer
	Transfusion-transmitted infections
	Neurodegenerative disease associated with infectious agents (e.g., prion disease)
	Chronic liver and kidney disease
	Transplant recipient
	Registered as opted out of organ donation

BASIC PROTOCOL STEPS FOR UNCONTROLLED DONORS

Several countries have published current protocols for uncontrolled NHBD donation [9, 16, 22–23].

At each of the stages of this type of donation, organizational and technical challenges may arise, and in order to best overcome them, a universal protocol for dealing with uncontrolled donors, adapted from the publication of Ortega-Deballon et al. is presented [24].

Step 1: Determination of conditions forwithholding resuscitation or discontinuing it if unsuccessful

The possibility of performing uncontrolled organ donation is considered only when resuscitative measures are not indicated or when they are performed but have no effect. Emergency medical personnel do not perform resuscitative measures when there are signs of obvious human death (decapitation, rigor mortis, etc.). Patients with signs of apparent death are not considered as uncontrolled donors.

Among the patients who undergo resuscitation measures, there are a number of those who have refractory CA, where it is inevitable that resuscitation should be discontinued because of lack of effect. In most jurisdictions, the decision to terminate resuscitation is taken by the health care provider based on existing national guidelines [25–27].

Organizational system configurations when dealing with uncontrolled donors to support steps 2 to 4

Stages 2 to 4, the names of which are presented in Figure, are considered within the framework of outlining possible organizational systems in place at hospitals dealing with uncontrolled organ donors.

The desire to minimize warm ischemia time (the period between CA and the beginning of preservation) in the donor is associated with unfavorable outcomes of transplantation from donors with a long warm ischemia time. At the same time, this circumstance should not be reflected in the duration of resuscitation measures performed on the patient. In this regard, it is extremely important to use two separate medical teams, one to perform resuscitative measures and terminate such measures where ineffective and subsequent certification of death, and the other to perform activities related to organ donation, starting work only after the patient's death has been certified by the resuscitation team [28–29].

System Configuration 1: A single pre-hospital team to provide resuscitation and transition to organ preservation.

A prerequisite for this configuration is national legislation on presumption of consent for deceased donation with a functioning donation opt-out register. A medical team of paramedics (similar to a paramedic in Russia) and doctors, having terminated resuscitation measures

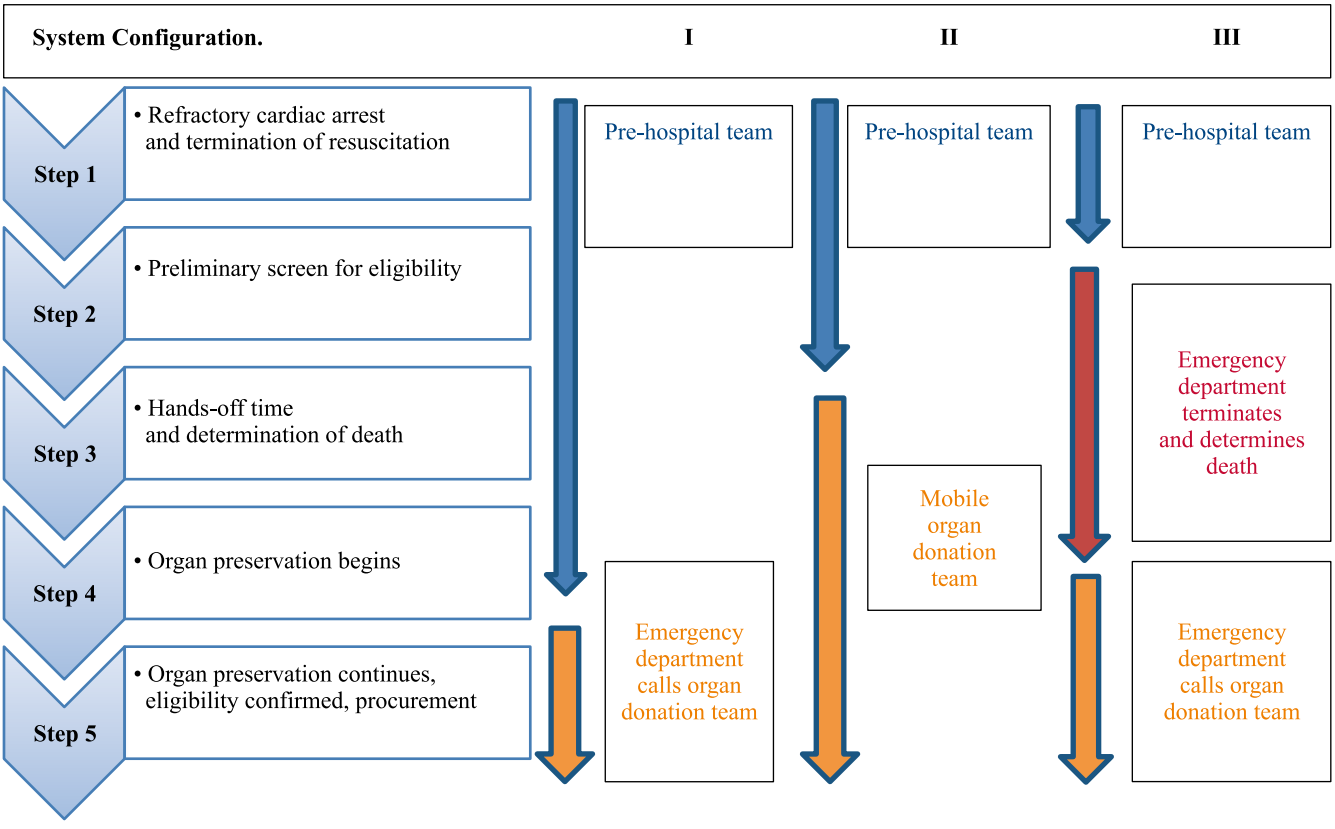

Fig. Basic protocol steps for uncontrolled DCD (Maastricht I and II) [9]

if such were unsuccessful and having ascertained the patient's death in out-of-hospital conditions, at the stage of transportation to the hospital, receive information about the patient from the opt out register, contact the relatives of the deceased and initiate measures for organ preservation [30]. When a potential uncontrolled donor is transported to the hospital, the prehospital team performs continuous cardiac massage with a mechanical device and oxygenation as the initial stage of organ preservation; in some cases, femoral cannulation is performed for perfusion preservation, which continues in the hospital [9].

System Configuration 2: One prehospital CPR team, and a mobile organ donation team.

In actual practice, this configuration is rarely seen. The New York Protocol (2011) is most often cited as being based on this configuration. Within 2 minutes of completing CPR, the prehospital medical team notifies the mobile organ donation team. In the New York experience, the paramedic team called the donation team 9 times, but none of the patients who died were registered in the organ donor registry, and only 4 met the inclusion criteria. No organ removal was performed and the program was discontinued. The main difficulties of the configuration under consideration are the transition from completion of CPR to organ preservation in out-of-hospital settings, when organizational and technical resources are limited and medical personnel often interact with relatives of the deceased person who are under strong emotional stress [9, 23, 31].

System Configuration 3: Continuation of pre-hospital CPR and transportation to the hospital for decision making and organ donation team involvement.

Previously, there was a publication by Scottish authors that presented this configuration. But it is difficult to evaluate its effectiveness because, according to the experience described in the report, the work with donors was conducted only on weekdays during standard business hours, which significantly limited possible donation in persons admitted with OHCA [32]. Specialists from Pittsburgh presented a study using the configuration under consideration. Of 50 patients who died in a hospital emergency department after an OHCA, 6 possible donors were identified, of which 4 organs were obtained from 2 donors [33]. Both programs were discontinued due to its ineffectiveness [9].

However, this configuration has been used very successfully for many years in countries with high rates of uncontrolled donation – Spain, France, Italy.

In Moscow, with the beginning of the uncontrolled donation program, a similar configuration is used, when, in the hospital, the medical staff of the shock ward continues resuscitation measures initiated at the prehospital stage; if they are unsuccessful, the patient is declared dead and the transplant coordinator calls the organ donation team [14].

Declaration of death and initiation of organ preservation in OHCA donors should be performed in the emergency unit [34], which is the most acceptable from organizational and ethical points of view.

Step 3: Cessation of resuscitative measures (hands-off time, non-touch period) and declaration of death

The WHO guidelines on stages of human circulatory death[3] emphasize a period between withdrawal of resuscitation and death, referred to as the "hands-off time" or "non-touch period", i.e. a period of refraining from any manipulation and rather observing the patient for 2–5 minutes after withdrawal of treatment or the patient's will to opt out of resuscitation, and 7 minutes if CPR has been fully performed. A longer follow-up period in cases with prior CPR is associated with increased likelihood of autoresuscitation or resumption of spontaneous cardiac activity after withdrawal of resuscitation. A systematic review by K. Hornby et al. states that resumption of cardiac activity in donors after circulatory arrest did not occur after a 7-minute "non-touch" period [35].

The inclusion of a "non-touch" period in the standardized protocol for working with uncontrolled donors differentiates the work of medical and donation teams, which increases confidence in both the provision of medical care and in the organ donation process, making the work of doctors more organized and comfortable [36].

WHO has published international guidelines on the definition of death, including definitions of brain death and cardiocirculatory death [37]. "Brain death" is defined as irreversible cessation of all brain function, and "circulatory death" is defined as cessation of circulatory function.

The WHO-recommended definition for determination of circulatory death at the current stage of medical development is as follows: "Circulatory death is the absence of any circulatory function after a hands-off time interval of 2 to 5 minutes without any preceding cardiopulmonary resuscitation or 7 minutes when preceded by any resuscitation" [37].

The minimum acceptable standard for declaring cessation of circulation (blood flow) includes:

1. No palpable pulse.
2. No breathing.
3. No heart sounds.
4. No breathing effort or chest movements.
5. No pulse pressure on non-invasive blood pressure measurement and no pressure wave on invasive blood pressure measurement.

[3] It refers to human death resulting from circulatory arrest.

6. Coma and fixed dilated pupils.
7. No electrical asystole required (pulseless electrical activity is acceptable).

Step 4: Preservation of donor's organs

If a possible OHCA donor is medically eligible, the next step after death has been declared is preservation of organ for transplantation. Whether pre-hospital or hospital-based organ preservation measures should be initiated depends on the current protocol for dealing with OHCA donors. Ethically and medically, the optimal place to initiate organ preservation is in the emergency department of the hospital where the OHCA patient was pronounced dead. It is better if the manipulations directly related to organ preservation are performed by the organ donation team, and the method of preservation depends on the choice of the specific donation program. From the experience of most programs (Spain, France, Netherlands), the best method of preservation of organs from OHCA donors is normothermic regional perfusion (abdominal, thoracic) or total (thoracoabdominal), when after death has been confirmed, blood circulation in the organs is restored by extracorporeal membrane oxygenation (ECMO), for which the donor's femoral (or other) vessels are cannulated and normothermic blood circulation is provided in the veno-arterial ECMO format [9, 14].

KIDNEY TRANSPLANTATION FROM UNCONTROLLED DONORS

The main concern relating to kidney transplantation from uncontrolled donors is the high incidence of primary nonfunction, ranging from 7–8%. Notwithstanding, the reported 1-year graft survival figures are equivalent to those from expanded criteria donors (ECD), and 10-year graft survival of between 72% and 82% was reported in the two single-center series with longest reported follow-up period [38].

In a study by J. Demiselle et al., delayed graft function (DGF) was more common in the group of recipients who received kidneys from uncontrolled DCDs (66%). However, at 3 months after transplantation, graft function was comparable to the group of recipients who received kidneys from ECDs. The authors argue that the use of normothermic regional perfusion in the uncontrolled DCD group was associated with a lower risk of DGF and with a better graft function at 2 years post-transplantation compared to *in situ* cold perfusion DCD group [39].

A study by W. Hanf et al. found no differences in glomerular filtration rate between grafts from uncontrolled deceased donors after cardiac arrest (uDDCA) and expanded criteria brain-dead donors (ecBDD); histologic evaluation showed no differences with respect to interstitial lesions [40].

Thus, the outcomes of kidney transplantation from uDDCA are comparable to those of transplantation from ecBDD [39], and modern preservation techniques such as normothermic regional perfusion (*in situ*) and machine perfusion (*ex vivo*) contribute to these outcomes.

LIVER TRANSPLANTATION FROM UNCONTROLLED DONORS

In world practice, liver transplants from uncontrolled donors have been performed, although the outcomes are less favorable than in kidney transplantation. In a study by Fondevila et al. [41], 34 (9%) liver transplants were performed from 400 potential uncontrolled non-heart-beating donors in Spain, with 236 (59%) and 130 (32%) livers turned down due to absolute and relative contraindications to donate, respectively. One-year recipient and graft survivals were 82% and 70%, respectively (median follow-up 24 months).

In a prospective study involving 60 adult liver recipients, 20 of whom received livers from donors with irreversible CA (Maastricht II) and 40 from brain-dead donors, [42] the rate of primary nonfunction was found to be 10% (n = 2) and 2.5% (n = 1), respectively. One-year cumulative patient survival was 85.5% in recipients who received liver transplants from uncontrolled non-heart-beating donors and 87.5% from brain-dead donors (P = 0.768).

CONCLUSION

Organ donation after OHCA, which first became possible in 1986 with the beginning of the practice in Barcelona (Spain), has not lost its relevance today. Mortality from OHCA continues to be high, reaching 90.0%, and such patients are saved using the most advanced methods of circulatory resuscitation, including external chest compression devices and ECMO. If resuscitative measures fail, the same medical devices allow to restore blood flow in organs and subsequently use organs for transplantation. Additional perfusion of organs obtained from uncontrolled OHCA donors, but already performed in *ex vivo* conditions on special devices, allows to make an objective assessment of the suitability of organs for transplantation, and to correct ischemic injuries sustained in the process of dying.

There is no doubt that this type of donation is extremely relevant for Russia. Large Russian megacities have a well-equipped and organized emergency medical services (EMS) system. Ambulance crews immediately go to OHCA patients and, placing the patients under continuous external cardiac massage and artificial ventilation, take the patients to the hospital, where resuscitation and other medical measures that are aimed at saving the patient's life are continued. If the resuscitation measures are unsuccessful, it is necessary to ensure the possibility of moving on to the process of organ donation for transplantation.

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DEVELOPMENT OF AN EXPERIMENTAL TECHNIQUE FOR ORTHOTOPIC LEFT LUNG TRANSPLANTATION IN A RABBIT MODEL

V.K. Bogdanov, I.V. Pashkov, Ya.S. Yakunin, E.A. Stakhanova, A.Z. Guluev, A.P. Kuleshov, O.Yu. Esipova, N.V. Grudinin

Shumakov National Medical Research Center of Transplantology and Artificial Organs, Moscow, Russian Federation

Objective: to develop, master and evaluate the efficiency of an isolated lung transplantation (LT) technique on a rabbit animal model using Perfadex Plus® solution for cold static storage. **Materials and methods.** Scottish Giant rabbits (n = 20) were used in this study and divided into two groups: donors and recipients. Donor lungs were preserved with Perfadex Plus® solution and stored for 6 hours at 4 °C. Recipient animals underwent unilateral orthotopic left LT. The postoperative follow-up period was 24 hours. Laboratory and instrumental control with assessment of blood gas composition, lactate level, ventilation parameters, and central hemodynamic parameters, was performed during the follow-up. Chest X-ray in direct projection was performed twice, and at the end of follow-up, material was taken for histologic examination. **Results.** We obtained a high oxygenation index in the post-transplant period (>350 at $p < 0.023$), as well as physiological indicators of lactate (3 ± 0.3 mmol/L at $p < 0.002$) and peak inspiratory pressure (15 ± 1 cmH_2O, $p < 0.001$). Radiological examination showed no radiological signs of severe primary graft dysfunction in all cases (mean RALE score 1), which was confirmed by histological studies. **Conclusion.** Left LT in rabbits is possible, the LT technique on a biological rabbit model using Perfadex Plus® solution is valid and efficient with the achievement of satisfactory gas exchange, ventilation and metabolism parameters.

Keywords: transplantology, lung transplantation, rabbit lung transplantation model, preservative solution, cold static storage.

INTRODUCTION

Currently, there are several unsolved issues about transplantation and perfusion of donor lungs. Some of them are ischemia-reperfusion injury (IRI), lack of highly effective antioxidant protection, and lack of a cheap and easily reproducible experimental model for scientific research, whose results can be extrapolated to humans [1–3]. Animals are the preferred experimental model for studying the respiratory system similar to that of humans. The choice of an optimal animal model is very important and should have the ability of the respiratory system to respond pathophysiologically to stressful conditions and the response should be close to how the human respiratory system responds to similar triggers. Consequently, the animal model should mimic human lung conditions in response to injury and surgical intervention at the clinical, biological, physiological and pathological levels [4–6].

Undoubtedly, an experimental model of large animals is preferable for simulating the pathophysiology of LT because their structural and functional features and anatomical characteristics are close to those of humans. However, the complexity of maintenance, high cost of a single study and species specificity of each large animal – dog, pig and sheep – dictate the need to search for an optimal animal. Based on international reports, rabbits are phylogenetically closest to large mammals [7, 8]. Immunological response to solid organ allotransplantation in these animals is close in specificity to similar manifestations in humans. An equally important aspect in choosing an animal LT model is the possibility of adequate monitoring of vital functions during the follow-up period, as well as the potential blood volume for hematological studies [9].

Therefore, we carried out work aimed at creating a reproducible orthotopic left LT model in rabbits. This study describes the peculiarities of anesthetic therapy and surgical technique both on the donor and the recipient, and it validates the technique as a whole.

Purpose of the present study: To develop and optimize orthotopic left-lung transplantation in an experimental rabbit model in order to assess the reproducibility of the animal model.

Corresponding author: Vladimir Bogdanov. Address: 1, Shchukinskaya str., Moscow, 123182, Russian Federation. Phone: (900) 110-42-41. E-mail: bogdanovv@bk.ru

MATERIALS AND METHODS

Male Scottish Giant rabbits weighing 4.5 kg to 5 kg were used in the study. The animals were divided into 2 groups: donors (N = 10) and recipients (N = 10). The experimental program was approved by the Committee on Biological Safety and Bioethics, Shumakov National Medical Research Center of Transplantology and Artificial Organs. The work was carried out in compliance with the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes and Directive 2010/63/EU.

The experimental design included removal of donor lung, static hypothermic preservation for 6 hours and orthotopic left LT procedure. The follow-up period to assess the severity of IRI and the effectiveness of the preservative properties of the experimental solution lasted for 24 hours. At the end of the experiment, the animal was removed from the experiment by exsanguination.

Donor lung removal

As part of the preoperative preparation, the animals were sedated with zolazepam (Zoletil 100, Virbac, France) subcutaneously at a dose of 50 mg. Under aseptic and antiseptic conditions, intravenous catheter Vasofix Certo 22G (BBraun, Germany) was inserted into the marginal ear vein, the catheter was fixed with a patch. Intravenous injection of atropine 0.3 mg and dexamethasone 2 mg was used as premedication. The donors were anesthetized with zolazepam 10 mg/kg, propofol (Fresenius Kabi, Germany) at a dose of 25 mg, followed by a combination of inhaled anesthetic Isoflurane (Baxter, USA) 1.5% vol. Tracheal intubation was performed by direct laryngoscopy using a size 4 endotracheal tube with an inflatable cuff. After correct intubation has been verified, rocuronium bromide solution (Fresenius Kabi, Germany) was administered at a calculated dose of 10 mg.

Artificial ventilation was performed using anesthesia machine WATO EX-65 Pro vet (Mindray, China) in volume controlled ventilation (VCV) mode with the following parameters: ventilation volume (V), 50 mL; respiratory rate (RR), 35/min; peak inspiratory pressure (P_{peak}), 17 cmH_2O; positive end-expiratory pressure (PEEP), 3 cmH_2O; inhalation/exhalation ratio (I:E), 1:1; fraction of inspired oxygen (FiO_2), 0.6; end-tidal carbon dioxide pressure ($EtCO_2$), 40 mmHg. Vital functions were monitored using the ePM 12M Vet device (Mindray, China) with an average heart rate of 170 beats/min, SpO_2 98, non-invasive blood pressure (NIBP) 90/45 mmHg. Tramadol (Tramvet, Russia) 25 mg intravenously was used for analgesia. Hemodynamic maintenance was provided by intravenous injection of potassium and magnesium aspartate (Panangin, Gedeon Richter, Hungary) 10 mL/hour and norepinephrine 100 ng/kg through a syringe dispenser.

Surgical access was performed via median sternotomy. After achieving hemostasis and the pulmonary artery trunk mobilized, heparin sodium 5000 U was injected intravenously with an exposure time of 3 minutes, followed by selective injection of alprostadil (Vasaprostane, IDT BIOLOGIKA, Germany) at 10 mcg dose. The pulmonary artery was cannulated with a 14 G intravenous catheter and antegrade pneumoplegia was initiated with Perfadex plus® at 40 °C and 60 mL volume through a syringe dispenser at a rate of 500 mL/hour and exposure time of 7–8 minutes. Mechanical ventilation (MV) parameters were varied: V, 25 ml; RR, 20/min; P_{peak}, 11 cmH_2O; PEEP, 5 cmH_2O; I:E, 1:1. Upon completion of perfusion of the preservative solution, the heart was removed first, and the pulmonary ligaments were crossed. The trachea was mobilized throughout its entire length and then tied with a silk ligature at the height of inspiration and crossed. Upon completion of organ removal, the lungs were placed in a sterile bag with subsequent static hypothermic preservation in an insulated container for 6 hours until it was implanted in the recipient.

Orthotopic left lung transplantation

Anesthetic management of the recipient animal differed from the donor stage in that an intravenous catheter Certofix Mono Paed S110 22G (BBraun, Germany) was placed in the marginal ear vein and a catheter CK-FLON 26G (India) was implanted in the middle artery of the ear to monitor invasive blood pressure. After the recipient has been anesthetized and vital functions monitored, the animal was laid on its right side, the surgical field was treated with an antiseptic and isolated with sterile surgical linen.

Surgical access was performed by left-sided thoracotomy in the fourth intercostal space with resection of the fifth rib. The wound edges were widened with a retractor, and after achieving hemostasis, we proceeded to mobilize left lung root elements. The pulmonary artery, main bronchus and separately the pulmonary veins were isolated from the surrounding tissues by blunt and sharp methods. After isolation of all vascular structures, the pulmonary artery, superior and inferior pulmonary veins were ligated. Last of all, the left main bronchus was ligated and crossed 0.5 cm from the tracheal bifurcation (Fig. 1).

Upon completion of pneumonectomy, 150 mg heparin sodium solution was administered, and single-lung ventilation was initiated with the following parameters: V, 25 mL; RR, 55/min; P_{peak}, 16 cmH_2O; PEEP, 5 cmH_2O; I:E, 1:1; FiO_2, 0.8; $EtCO_2$, 36 mmHg. Ninety minutes after the onset of anesthesia, 30 mg zolazepam and 25 mg tramadol were administered intravenously. Sedation throughout the operation was performed with isoflurane 1.0 vol.%. Hemodynamic maintenance was provided by intravenous injection of potassium and magnesium aspartate, 5–10 mL/hour, and norepinephrine

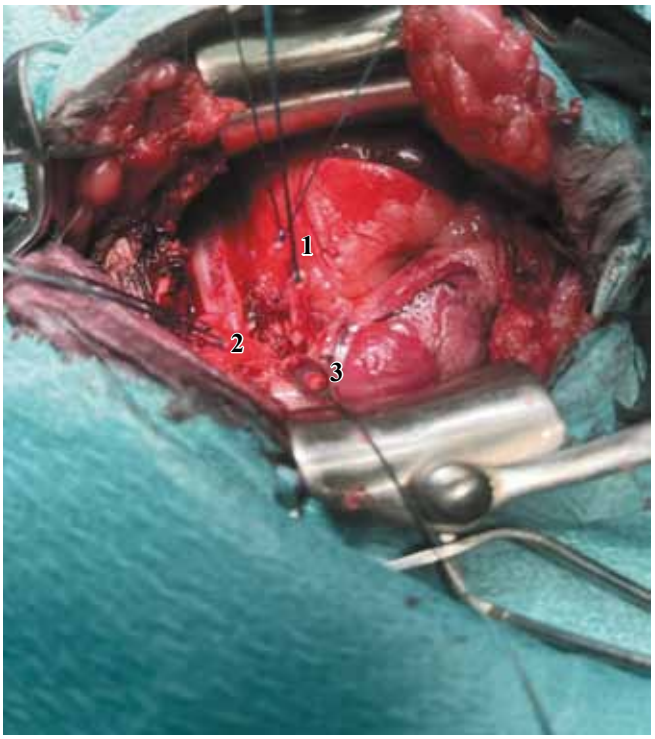

Fig. 1. Condition after pneumonectomy of the left lung. 1, pulmonary vein; 2, pulmonary artery; 3, main bronchus

100–460 ng/kg. Blood gas and electrolyte composition was studied every 30 minutes using an EDAN Blood Gas Analyzer (Edan Instruments, China); samples were taken from a catheter in the ear artery. Electrolyte and metabolic disorders were corrected symptomatically.

In order to conveniently apply a clamp to the left atrium, the pericardium was opened transversely from the mouths of pulmonary veins. Then, a vascular clamp was applied to the pulmonary artery and the left main bronchus. In order to avoid kinking of vascular anastomoses, a bronchial anastomosis was applied first. The bronchial anastomosis was formed by continuous wraparound suture using a PDS 6/0 thread. Anastomosis of the pulmonary artery was performed with continuous wraparound suture using a Prolene 8/0 thread. Atrial anastomosis was performed last. A Satinsky vascular clamp was applied to the pulmonary veins with maximal capture of the free wall of the left atrium. The pulmonary veins were crossed, and a single left atrial cuff was formed by angular vascular scissors, the anastomosis was performed with a Prolene 7/0 thread. After anastomosis formation was completed, graft reperfusion was initiated. After implantation of the donor lung, Methylprednisolone (Pfizer, Belgium) was administered intravenously at a dose of 50 mg before starting blood flow. Ventilation was resumed, the clamp was first removed from the pulmonary veins thereby initiating retrograde perfusion; after the graft was filled and blood appeared from the untied suture line on the pulmonary artery, the suture was tied. Next, the clamp was removed from the pulmonary artery, thereby completely resuming blood flow in the graft. The recruitment maneuver was performed automatically with P_{peak} at 30 cmH_2O.

Radiologic study

A straight chest x-ray was taken for a radiological examination. In order to assess IRI severity, the RALE (Radiographic Assessment of Lung Edema) scale was adopted [10]. The peculiarity of this technique for measuring the severity of lung lesions lies with its universality and simplicity, as well as validity of its application in animals. Since only the left lung was transplanted, the RALE score was assessed unilaterally. The lung was visually divided into two quadrants, and each quadrant was assigned a consolidation score from 0 to 4 to quantify the degree of alveolar opacities based on the percentage of the quadrant with opacities, and a density score from 1 to 3 to quantify the total density of alveolar opacities, except when the consolidation score for that quadrant was 0. Because consolidation is a process requiring more than 24 hours of follow-up and its assessment is difficult with short follow-up times, its value was conventionally taken as 1.

Left lung lesion was computed using the formula: Upper lobe consolidation score × upper lobe density score = Q1; Lower lobe consolidation score × lower lobe density score = Q2; Q1 + Q2 = total RALE score.

Statistical analysis was carried out using the StatTech v. 3.1.10 software (StatTech LLC, Russia). Quantitative indicators were evaluated for conformity to normal distribution using the Shapiro–Wilk Test (number of subjects less than 50). Quantitative indicators having normal distribution were described using arithmetic mean (M) and standard deviations (SD), 95% confidence interval (95% CI) limits. One-factor analysis of variance with repeated measures was used to compare three or more related groups for a normally distributed quantitative trait. Statistical significance of changes in an indicator in dynamics was assessed using Pillai's Trace (Pillai's Trace). Posterior analysis was performed using paired student's t-test with Holm correction. Results were considered statistically significant at $p < 0.05$.

RESULTS

Dynamics of blood gas composition and lactate levels, peak inspiratory pressure parameters in recipient animals

The main parameters studied during the left LT were peak inspiratory pressure in the recipient animal (Fig. 3), lactate levels (Fig. 4), as well as the calculated indicator – PaO_2/FiO_2 ratio for arterial blood from arterial catheter (Fig. 2). All investigated cases showed satisfactory graft function at control assessment of gas composition, peak airway pressure and lactate directly from the pulmonary vein.

When studying the oxygenation index, the indicators were found to have moderately decreased (345 ± 32, from 283 to 385) in all cases after implantation and at 1 hour. After graft reperfusion and at 24 hours of follow-up, oxygenation index values >350 were observed in five cases (N = 5). However, when assessing the statistical population, mean oxygenation index was found to be high at 3 hours of follow-up.

The dynamics of changes in peak inspiratory pressure was a significant indicator for assessing the functional status of the donor lung, taking into account the fact that MV parameters were selected individually. At the end of

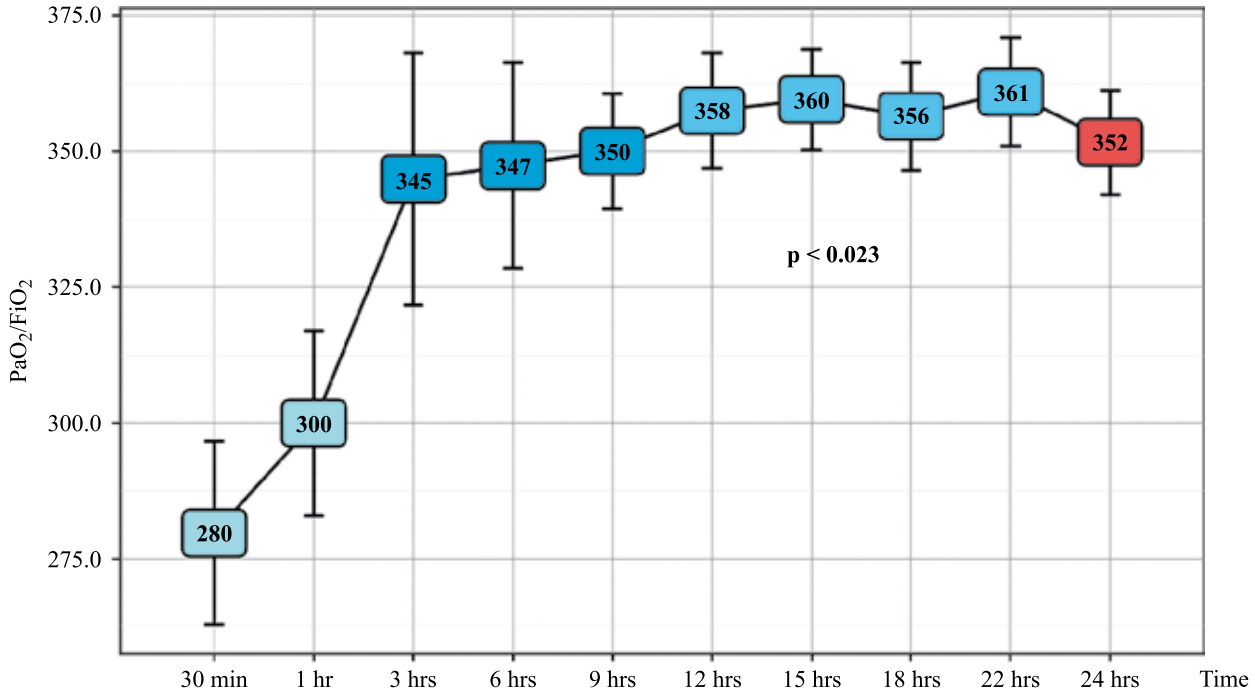

Fig. 2. Dynamics of oxygenation index after left lung transplantation. The graph shows mean values, vertical lines indicate standard deviations, p is statistical significance

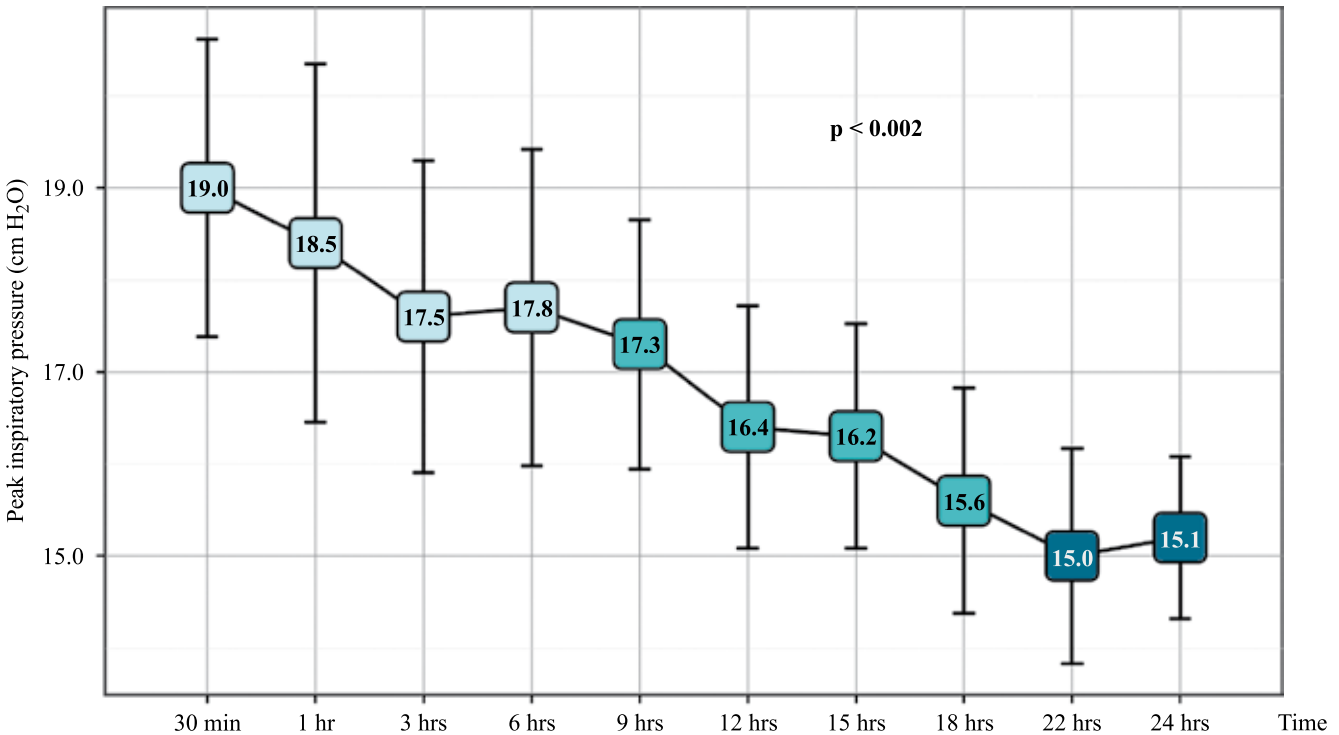

Fig. 3. Dynamics of changes in peak inspiratory pressure after left lung transplantation. The graph shows mean values, vertical lines indicate standard deviations, p is statistical significance

the follow-up, peak inspiratory pressure was low during the statistical population assessment: 15 ± 1 (from 14 to 16) cmH_2O, which indicated preserved graft function and no severe interstitial edema.

The dynamics of lactate parameters reflected the severity of IRI after transplantation, as well as the correctness of MV and adequate warming of the animal. When evaluating the statistical population, it was noted that lactate levels exceeded 8 mmol/L in only two cases, but at the end of the follow-up period, the levels remained at the physiologic level, 3 ± 0.3 mmol/L.

Results of the study of gas parameters, peak inspiratory airway pressure and lactate levels after transplantation at 10 time points indicated effective gas transport and satisfactory functional status of the donor lungs in all cases.

Radiologic studies

Radiologic examination was performed twice during the follow-up period in all cases. The results are presented in Table.

Radiological studies showed that lung grafts had signs of primary graft dysfunction in all cases. However, at the end of the follow-up period, significant signs of IRI regressed in all cases.

It should be noted that, according to reports, this is the first experience of using the RALE scale to assess the severity of donor lung injury in an experiment in rabbits. This suggests that the significance of IRI assessment can be low when examining and describing radiographs.

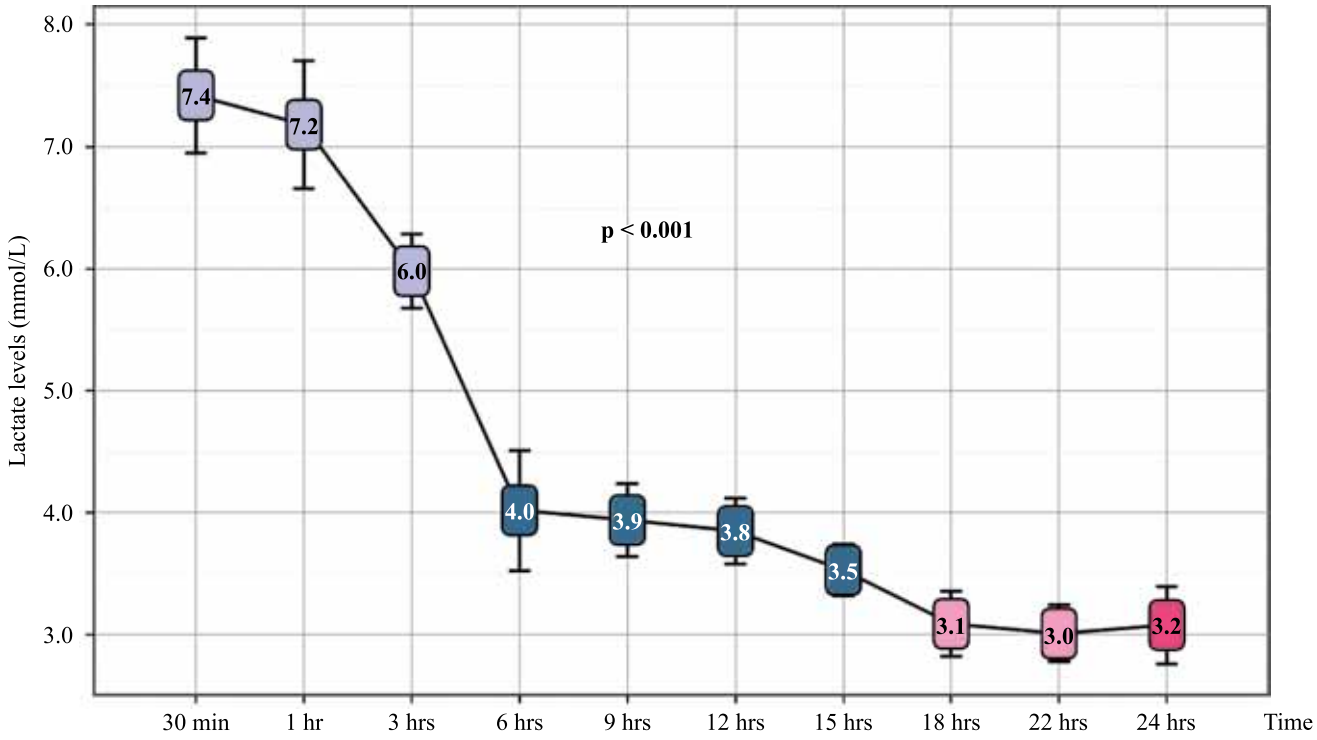

Fig. 4. Dynamics of changes in arterial blood lactate levels after left lung transplantation. The graph shows mean values, vertical lines indicate standard deviations, p is statistical significance

Table

Dynamics of the radiologic picture after left lung transplantation

	RALE Score after transplantation	RALE Score at 24 hours
Recipient 1	2	1
Recipient 2	1	1
Recipient 3	1	1
Recipient 4	2	2
Recipient 5	2	2
Recipient 6	1	1
Recipient 7	2	1
Recipient 8	2	1
Recipient 9	2	2
Recipient 10	1	1

Histological studies after transplantation

Histologic specimens were evaluated at 100× magnification (Fig. 5, a) and 200× magnification (Fig. 5, b) over the entire specimen area in each case.

A morphological study at 24 hours after implantation of the donor lung showed no difference in the microstructure of the lung parenchyma in the compared samples. Thus, in all cases, the pulmonary parenchyma architectonics were preserved, the alveolar-capillary barrier was intact, interstitial edema was moderate, functional structures were preserved, and there were no disseminated hemorrhage sites.

DISCUSSION

The LT procedure has a long history. Back in the last century, Soviet scientist Vladimir Demikhov attempted LT in an experiment on dogs. A significant contribution to the development of this area was made by French surgeon and biologist Alexis Carrel, who provided the formation of the basic principles of the technique. It is noteworthy that every scientist in world history has engaged in scientific research in experimental studies on animals with great respect [11]. Animal research facilitates the acquisition of experience and knowledge that would be implemented in clinical practice with great precision and outstanding results. There is still a need to find a universal animal model of LT. However, today humanity is closer than ever to perfection and professionalism, as well as to a deep understanding of solutions to combat IRI [12].

Although there are various experimental animal models of LT, each has its own peculiarities, strengths and weaknesses. The model of orthotopic left LT in rabbits is surgically and anesthesiologically challenging. The low weight of the animal requires a very competent anesthetic approach that is comparable to that of pediatric cardiac anesthesiologists. Constant monitoring of blood gas and electrolyte composition is necessary for early correction of metabolic disorders, and the possibilities of infusion therapy are severely limited due to the direct effect of infusion volume on postoperative interstitial edema and gas transport function of the blood. From a surgical point of view, the main challenges in working with the rabbit model are related to the need for microsurgical skills. Thin vascular walls, fragile tissue structures, and anatomical features present challenges for the operator.

Despite the abundance of biochemical, morphological and functional indicators for assessing the status of donor lungs in a clinical organ transplantation program, there is no more objective and statistically reliable criterion that characterizes the functional status of the graft than the oxygenation index. Thus, when studying this index within the framework of this work, high oxygenation index was obtained, which, together with the histological picture and the results of radiological studies, as well as ventilation parameters in the early postoperative period and the dynamics of changes in lactate levels, indicates the high efficiency of the technique of orthotopic left LT on a rabbit model at standard ischemic periods [13].

CONCLUSION

The experimental study showed that orthotopic left lung transplantation can be performed on an experimental rabbit model. The model has shown its efficiency and reproducibility. Certainly, the economic feasibility of this model looks more attractive in contrast to the use of large laboratory animals. The proposed experimental model will expand the arsenal of research teams that are dealing with LT problems.

The authors declare no conflict of interest.

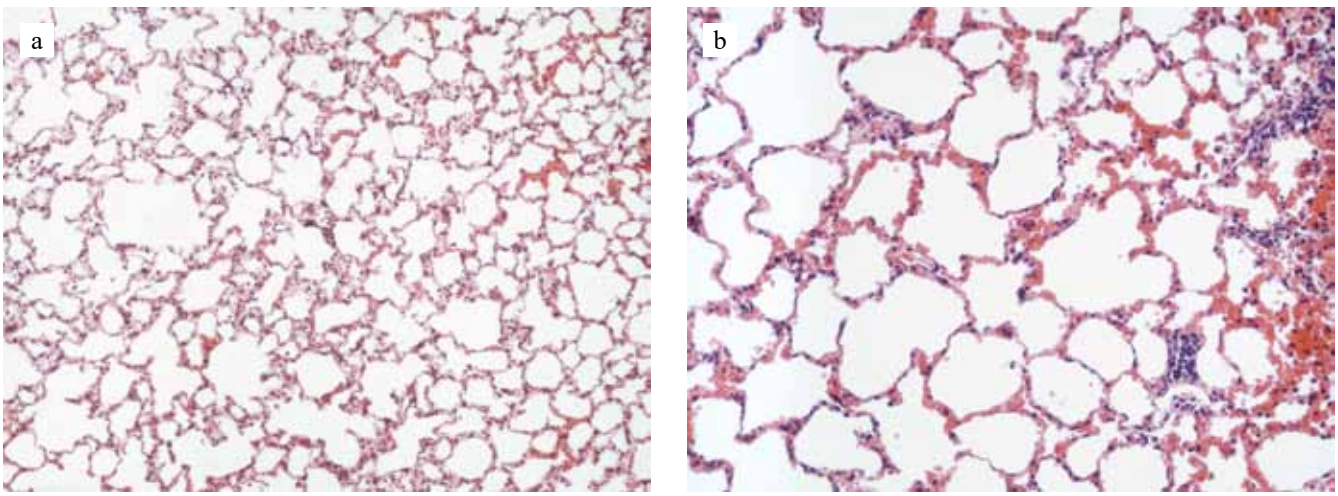

Fig. 5. Morphological study results: a, histological picture of donor left lung parenchyma at 24 hours after transplantation; 100× magnification; b, histological picture of the donor left lung parenchyma at 24 hours after transplantation; 200× magnification

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ORGAN DONATION AFTER EUTHANASIA. REVIEW AND CRITICISM OF FOREIGN PRACTICE

O.N. Reznik[1–3]

[1] St. Petersburg Research Institute of Emergency Medicine, St. Petersburg, Russian Federation
[2] Pavlov University, St. Petersburg, Russian Federation
[3] Institute of Philosophy, Moscow, Russian Federation

This paper analyzes the problem of euthanasia, gives the history of this phenomenon, presents traditional ethical arguments for and against this practice, critically evaluates the practice of organ donation after euthanasia or euthanasia as a consequence of organ donation as established in some countries of the European Union, the US and Canada. The current status of this controversial practice is assessed.

Keywords: euthanasia, organ donation, organ donation after euthanasia.

INTRODUCTION

Despite the emerging disengagement between Russia and the West, scientific ideas, their interpenetration and cooperation among professionals have always remained a priority of scientific life, especially in the field of high medical technologies, which, of course, includes transplantation medicine. An example of such exchange of ideas is the West's implementation of Sergei Brukhonenko's discoveries in the field of artificial blood circulation and the practice of organ "revitalization", as well Vladimir Demikhov's surgical techniques for organ transplantation [1]. In turn, the Russian medical community adopted the concept of brain death 25 years after the international recognition of the protocol for its establishment according to the Harvard Medical School criteria [2]. The interpenetration of ideas is expedient while preserving the ethical basis of transplantation technology, regardless of the cultural codes of different countries and civilizations.

The philosophical, ethical, and moral foundations of providing transplant care are as important as surgical technologies. The following problems are well known to hinder systematic development of transplantation everywhere: the appearance in the patient/doctor relationship, for the first time in the history of medicine, of an additional subject – donor organs, which are "therapeutic means" for patients in need of transplantation. Obtaining donor organs is a difficult task not only in the context of surgical and other medical aspects, such as the work of multidisciplinary teams in the process of diagnosing brain death and organizing organ removal, but also in terms of everyday awareness activities with the society, with the general public. Gaining and maintaining the public's trust and confidence in transplant physicians is a major challenge. As a result of the above-mentioned problems, organ shortage remains the main obstacle to widespread dissemination of transplant care.

According to reports from the Global Observatory on Donation and Transplantation, presented in the annual Newsletter Transplant 2023, 102,090 transplants were performed worldwide, and 361,197 patients were on the waiting list (by the example of kidney transplantation, information from reports of 86 countries, including China, India, Spain and the United States of America (USA), current as of December 12, 2022) [3]. The situation in Russian transplantation is regularly reported in the Registry of the Russian Transplant Society, and according to its 15th report, 2,555 transplants were performed in Russia in 2022 [4], and there were 8,378 people on the transplant waiting list (2019 data from the Report of the Chief Freelance Specialist Transplantologist of the Russian Ministry of Health, Sergey Gautier) [5].

Traditional sources of donor organs are brain-dead donors, donors after irreversible cardiac death, living donors of organs or organ parts (kidneys, part of liver, pancreas or lung).

However, some countries of the European Union, 8 states in USA and Canada, have recently developed some practices of overcoming the organ shortage crisis, which is ultra-liberal in nature. This specifically involves deceased organ donation after euthanasia (ODE) and including implementation of euthanasia as a consequence of organ donation.

Despite the ban on euthanasia in the Russian Federation, the ethical dangers of organ donation after voluntary assisted dying require careful analysis, since such well-developed methods of solving the problem of organ shortage can undermine the altruistic basis of this type of medical care.

This study of scientific literature is aimed at critically highlighting the historical perspective of euthanasia, tra-

Corresponding author: Oleg Reznik. Address: 9, Pereulok Grivtsova, Saint-Petersburg, 190031, Russian Federation.
Phone: (921) 935-51-91. E-mail: onreznik@gmail.com

cing its formation and transformation into an integral component of modern medical practice abroad, and assessing the controversial technology of ODE.

HISTORY OF THE DILEMMA OF VOLUNTARY ASSISTED DYING

Debates about the ethical justification of euthanasia – ending the life of a hopelessly ill or unbearably suffering person by his or her will – has been going on for almost three thousand years, dating back to ancient times. For example, Plato and Socrates considered it completely acceptable to end the lives of those who were not fit for it. The Stoics, from Zeno to Seneca, saw the act of voluntarily leaving life as a brave step and a noble alternative to passively accepting the dire consequences of a long illness or the actions of others. Aristotle argued that seeking death to escape from suffering or pain was an act of cowardice and therefore rejected euthanasia, while Pythagoras and his followers believed in the sacred nature of life and disapproved of any voluntary termination of life by man. Even then, *active* euthanasia was distinguished, which Hippocrates opposed directly in the text of his Oath ("*I will not give a lethal drug to anyone if I am asked, nor will I advise such a plan*"), and passive euthanasia, which he also supported, considering it acceptable if a doctor does not treat "*a patient over whom the disease has taken over*" [6–8].

The philosophical dilemma of euthanasia identified in ancient times is still relevant today, with a remarkable change in the tone of the debate about euthanasia: instead of abstract philosophical inquiries, today's bioethicists see this controversial practice as inextricably linked to modern medical activity.

In his definitive work, *Medicine and Care of the Dying: A Modern History*, public health expert Professor Milton J. Lewis thoroughly explores the attitudes of society and individuals toward death, tracing how, over the centuries, the phenomenon of human death gradually lost its sacred and religious basis, lost its status as a major ritual, and, as medicine evolved, turned into an increasingly routine phenomenon, until, finally, dying and death became a part of medical practice, and first became a medical routine, and then acquired a utilitarian component [9].

Euthanasia as *mercy killing* was first seriously advocated by a member of the British medical profession in 1901. Dr. C.E. Goddard, a public health doctor, not a clinician, who identified two classes of patients that need their lives to be terminated: the hopelessly ill and patients with hereditary pathologies, whose *useless* lives were proposed to be forcibly terminated. When philosopher Maurice Maeterlinck joined in this rhetoric, criticizing physicians for seeking to prolong patients' lives at any cost, practical medicine, represented by physician, writer, and pain management researcher Robert W. McCann, responded: "*abstract arguments about mercy killing of incurable patients are easily picked up when their author has no dealings with such patients and bears no personal responsibility for such actions – medicine is the art of healing, not dealing with death; ultimately, the prize is the death of the patient*" [9]. This excerpt illustrates how far apart practitioners and abstract thinkers can be when discussing techniques and practices not only indirectly affecting the patient but also directly related to his or her life and death. Let us consider in general the main arguments in favor of and against euthanasia.

Arguments in favor of euthanasia

In a 1994 review article, "*Euthanasia. Historical, Ethical, and Empirical Perspectives*", practicing oncologist, bioethicist, and euthanasia opponent, Ezekiel J. Emanuel, concluded that the arguments in favor of euthanasia have remained remarkably constant since they were first articulated in 1870. According to him, they are: 1. Individual autonomy – since there is no universal right to a dignified life for all people and everyone is free to live according to his or her own definition of dignity, society has no choice but to delegate to an individual the right not only to live with dignity, but also to leave life preserving dignity, i.e. to refuse treatment if he or she really wants to; 2. The principle of beneficence/charity, like individual autonomy, is one of the fundamental bioethical principles that equates to "do no harm" and enjoins the physician to act in the best interest of the patient by ending the patient's suffering when it comes to euthanasia; 3. Euthanasia is no different from withholding or withdrawing life-sustaining therapy in terminally ill patients, since the patient eventually dies in both cases. The only difference is that in euthanasia, the doctor himself administers the drug that ends the patient's suffering, and in this the proponents of euthanasia see no moral contradiction, noting, however, that the doctor's actions are fundamentally different in nature; 4. Finally, it is argued that the likely negative consequences of allowing euthanasia are abstract and too speculative to be the basis for public policy on the practice. For example, allowing euthanasia in the Netherlands did not lead to the expected fall in citizens' trust in medical professionals [10].

Arguments against euthanasia

Opponents of euthanasia, selecting counterarguments, turn to the already mentioned basic principles of bioethics. 1. For example, it is believed that individual autonomy does not justify euthanasia. Personal autonomy as an ethical principle is valid only in "ideal conditions", when it is assumed by default that someone's choices, decisions, beliefs and desires are not influenced from the outside, and he/she is not the object of manipulation and/or coercion [11]; 2. The principle of charity/beneficence is also not a sufficient reason for euthanasia, since neither physicians nor the health care system in general currently offer complete protocols for sufficient pain management in hopelessly ill patients, nor are there algorithms to relieve suffering sufficiently, and therefore it is prema-

ture to resort to euthanasia; 3. Opponents of euthanasia clearly distinguish, from an ethical standpoint, between "medical homicide", when a physician takes active steps towards ending a patient's suffering, and the termination of the physician's participation in the natural course of an incurable disease that will definitely lead to the patient's death eventually, considering as inappropriate any interventions and as acceptable any passive observation of the death of a terminally ill patient; 4. Finally, opponents of euthanasia consider its legalization as a "perilous public policy", which can have detrimental effects both on the doctor-patient relationship and on medical activity in general. In addition, legalization of euthanasia may undermine the compassionate and humanistic basis of care for the terminally ill, when instead of thoughtful control of the manifestations of the disease, the choice of "alleviating" the patient's suffering and "solving" the doctor's problems, up to the point of ultimately equating killing with healing, is made [10].

The "sloping plane" or "slippery slope" argument is often used when discussing euthanasia. The meaning was conveyed by Justo Aznar, Director of the Bioethics Observatory of the Institute of Life Sciences at the Catholic University of Valencia, in a recent article as follows: "*When a door is opened to give way to an issue with a significant bioethical burden, we know that it will go through it at that time. What we do not know is what will continue to go through that door over time and whether, at some point, what may go through will be ethically illicit.*" [12]. The above paper cites three questionable consequences of legalizing euthanasia: 1. Euthanasia techniques could be applied in non-terminal psychiatric patients who are not in unbearable suffering; 2. It could also be carried out in adolescents, children and neonates; 3. Involuntary euthanasia may be performed [12].

In the book "*Euthanasia, Ethics and Public Policy: An Argument Against Legislation*", the relevant chapter entitled "The Slippery Slope Arguments" begins with this definition: "… *the "slippery slope" is that if a proposal is made to accept A, which people do not agree is immoral, it should nevertheless be rejected because it would likely lead to B, which people universally or generally agree to be immoral*" [13]. The authors further conclude that euthanasia is unacceptable, providing empirical and logical arguments in favor of this position. If euthanasia is acceptable for the "hopelessly ill" who are in "unbearable pain" and who have also given "voluntary and informed consent" by expressing it in the form of a "last wish", then there is no obstacle to "relaxing" the law in the future by allowing those who are "non-terminally ill" but "in chronic pain", for example, "physical discomfort" or "existential crisis", because the autonomy of the individual "does not know" the conditional boundaries defined by the law, and the state, according to the authors, is not able to provide appropriate guarantees and/or make detailed recommendations to ensure the realization of socially acceptable "ideal euthanasia" [13]. The book "*Euthanasia and Assisted Suicide: Lessons from Belgium*" explores the possibility of legalizing voluntary withdrawal from life for people who are dissatisfied with life, without having a terminal illness or unbearable suffering, but do not want to continue living for subjective reasons. As an example, a case is given when a British citizen, "tired of life in modern society" turned to the Swiss organization "Dignitas", where she received medical assistance in dying (MAiD) [14].

As will be shown later, bioethical reflection has traditionally lagged behind medical progress, nevertheless managing to accurately predict the consequences of the "slippery slope".

CLASSIFICATION OF EUTHANASIA: FROM "MEDICAL SUICIDE" TO "DEATH WITH DIGNITY"

As an illustration, we present a classical classification of euthanasia and then trace the evolution of terminology.

1. Voluntary active euthanasia. Intentional administration of drugs or use of other medication leading to the death of a patient, which is carried out at the patient's explicit request and is done after obtaining fully voluntary informed consent;
2. Involuntary active euthanasia. Intentional administration of drugs or use of other medication leading to the death of a patient, which is carried out when the patient was capable but did not expressly request and/or did not give voluntary informed consent to the procedure, e.g. when not asked;
3. Non-voluntary active euthanasia. Intentionally administering drugs or using other medications leading to the death of a patient when the patient is incapacitated and therefore unable to request euthanasia, such as being in a coma or suffering from a psychiatric illness;
4. Termination of life-sustaining treatment (passive euthanasia). Refusing or terminating life-sustaining medical care to allow a patient to die;
5. Indirect euthanasia (indirect euthanasia). Administration of narcotic or other drugs to relieve pain at doses sufficient to depress respiration and cause the patient's death;
6. Physician-assisted suicide. The physician provides the patient with medication or expresses a willingness to intervene, realizing that the patient is thus planning to commit suicide [10].

The last paragraph is of interest, since it is the first time that direct involvement of medical professionals in ending the lives of patients is articulated. There is a characteristic change in the tone of the discourse on "physician-assisted suicide", which is characterized by a shift in emphasis from the potentially disturbing terms "suicide" and "homicide" towards medical *assistance* or *aid in dying*. As defined by the American Medical Association, physician-assisted dying is the "means and/or information" to facilitate the decision to end one's

life [15]. A publication in the pages of the Yale Journal of Biology and Medicine also cites synonyms such as: "right to die", "physician-assigned death", "death with dignity", abbreviations "AiD" ("Aid in Dying") and "MAiD" ("Medical Aid/Assistance in Dying"), which are read as "aid" or derivative of "attendance" and allow euthanasia supporters not only to talk about death, but also to participate directly in it, avoiding negative interpretation of their actions [16]. The authors, taking a neutral position, express concern about the implicit or unintended consequences of legalizing the practice of voluntary death.

First, it is the "suicide contagion" ("suicide infection"), a phenomenon first described by sociologist David Phillips in 1970, which boils down to the following: the suicide of a famous person is followed by a spike in suicides among ordinary members of society [17]. The case of Brittany Maynard, an American activist who suffered from the last stage of glioblastoma, promoted "assisted dying" and voluntarily passed away on November 1, 2014, at the age of 29, is cited as an example. In the months that followed, the number of "deaths with dignity" in Oregon, where euthanasia has been legalized since 1997 by the aptly named Death with Dignity Act [18], doubled [16]. The existence of such organizations as Death with Dignity National Center [19] and Dying With Dignity Canada [20] in the USA and Canada [19], respectively, reflects a targeted policy to popularize voluntary dying. For example, the homepage of the American resource welcomes the user with the slogan "We should all have the right to die with dignity", while the Canadian resource opens with the slogan "It's your life. It's your choice." These resources are not only informational in nature, but also suggest taking an active stance on voluntary death, literally offering to "fight" for the right to euthanasia.

Second, attention is being paid to the problem of clinical depression. Up to half of patients diagnosed with cancer and older adults considering voluntary death have evidence of a depressive disorder, yet they are not specifically screened for depression. These categories of people account for more than 70% of the total number of voluntary deaths in Oregon, with professional psychiatric or psychotherapeutic care offered to less than 5% of them [16]. Official sources provide the following information: for 2021, 383 euthanasia cases were reported in Oregon, and psychiatric evaluation was performed in only 2 patients [21]; in 2020, out of 188 patients, psychiatric care was offered to only 1 patient [22]. In Canada, where euthanasia is also legalized, there were 10,064 cases of voluntary death in 2021, with psychiatric consultation in only 644 of them [23]. Performance of euthanasia in non-terminal psychiatric patients is reflected in the practice of voluntary death by Belgian citizens [24]. In addition to Canada, Belgium, Switzerland, and Oregon, MAiD is now legal in the Netherlands, seven more US states (Washington, California, Montana, Colorado, New Mexico, Maine, Vermont; in Pennsylvania, Michigan, New York, and Massachusetts, bills on legalization of euthanasia are under consideration), Australia, Colombia, Luxembourg, Portugal, Spain, and New Zealand [25].

The above examples allow us to judge about the liberalization of the practice of euthanasia, when a sufficient reason for voluntary death may not be an incurable illness or unbearable suffering of a capable person, but psychiatric disorders, such as depression, schizophrenia, autism spectrum disorders, post-traumatic stress disorder, attention-deficit/hyperactivity disorder, anorexia [26, 27], as well as subjective dissatisfaction with life, "fatigue from it" [14]. A comprehensive review from the first roundtable on ODE, published in the pages of the American Journal of Transplantation in 2022, summarizes the indications for "voluntary medically assisted dying". These include: 1. Unbearable suffering with no prospect of improvement in the patient's condition, 2. Intolerable physical or mental suffering, 3. Persistent physical or mental suffering with no prospect of improvement in the patient's condition, 4. Intolerable physical or mental suffering that cannot be alleviated in a manner acceptable to the person experiencing it, 5. Suffering that cannot be alleviated in a manner acceptable to the person experiencing it (but not necessarily intolerable), 6. Persistent and intolerable physical or psychological suffering (without specifying the possible means of alleviating it), 7. Intense suffering with no available means of alleviating it. The review also specifies under which special medical conditions euthanasia is possible: 1. Incurable disease or condition, 2. Severe illness or disability, 3. Severe progressive illness, 4. Severe, progressive disease that will inevitably lead to the patient's death. However, special medical conditions are not required in the Netherlands, Belgium and Colombia [28]. This information indicates that there is no consensus in the expert community, and the questions remain open as to whether psychological and/or mental suffering can be objectively assessed, whether it is really impossible to cure a severe (not incurable) disease, whether palliative care resources are fully utilized for patients in need of it, and finally, whether civil society representatives are not manipulated by interested professionals.

The popularization of the concept of euthanasia in the Western society indirectly confirms the possibility of such manipulation and creates preconditions for rash and potentially dangerous decisions concerning issues of life and death by representatives of vulnerable social groups. For example, according to the World Health Organization, about 280 million people in the world have depression, more than 700,000 people die due to suicide every year, which, in addition, is one of the leading causes of death in 15–29-year-olds [29].

In connection with the above, the practices of post-euthanasia organ donation in Belgium, Canada, the Netherlands and Spain require special attention, where the humanistic idea of saving the lives of patients in need

of transplantation using the organs of those who have decided to die voluntarily may hide a utilitarian desire to radically expand the pool of available donor organs through ethically unsound methods that undermine the altruistic basis of transplantation.

A CRITICAL LOOK AT THE PRACTICE OF ODE

The pioneering experience of ODE belongs to Belgium, where the first such case was recorded in 2005, 3 years after its legalization. While in 2002, only 24 cases of euthanasia were recorded in Belgium, in 2022, 2,966 people voluntarily died, and a total of 30,185 people turned to this practice during the 10-year period [30]. The Netherlands is the leader in terms of the number of people who voluntarily die – euthanasia was approved for 82,963 people between 2002 and 2021 [26]. In Canada, euthanasia was legalized in 2016 and according to the Third Annual Report on Medical Assistance in Dying, which was "proudly" announced by the Minister of Health of Canada, 31,664 people voluntarily died in the country in 2021 [23]. It can be stated that the steady increase in cases of euthanasia in countries where it is legalized has become an epidemic in recent years.

Back in 2017, on the pages of the JAMA Network, a prominent researcher of ODE, Jan Bollen, suggested that about 10% of those who died after euthanasia could become organ donors and praised the prospects of promoting voluntary ODE as an effective way to combat the organ shortage crisis [31]. The practice is in its infancy, as evidenced by the modest rates of organ donation following euthanasia, with only 286 cases of organ donation following voluntary death until 2021 [28]. The unrealized pool of donors in the above countries, if we use the proposed estimate, was approximately 14,500 patients, which cannot be out of the sight of transplant specialists.

In 2016, a team of authors led by J. Bollen, published an article entitled "Organ Donation After Euthanasia: A Dutch Practical Manual" in the American Journal of Transplantation [32], which thoroughly describes the organizational basis of ODE. Special attention is paid to the ethical component of the procedure. The doctor is instructed to find out whether the patient's wish to die is not the result of pressure from the patient in need of transplantation, to try not to interfere with the altruistic intentions of the future donor; it is also indicated that the doctor is obliged to have a conversation about possible ODE in cases where the patient is not aware of such a prospect, appealing to the patient's right to self-determination as a special case of personal autonomy, and forming in him a noble image of the act of donation [32].

In 2023, JBI Evidence Synthesis published a fundamental analysis of the scientific literature on ODE, presented in two parts. The first part focuses on the ethical and legal bases of this practice (these have been discussed above). The second part is devoted to the existing clinical algorithms of ODE. The choice of the publication is characteristic, because "JBI", as follows from the description on its official website, is a global organization that promotes and supports evidence-based solutions and best practices that improve health and health care delivery [33]. It is assumed, apparently, that ODE is an evidence-based solution and the best practice to overcome the organ shortage crisis. So how is the practice of ODE actually implemented?

Organ donation following MAiD is a process that requires implementation of multistep procedures, and this process can occur in the hospital setting, in the patient's home, or start at home and be completed in a hospital setting. Patients who express a desire to become donors after euthanasia are usually hospitalized, as this allows specialists to continuously monitor the patient's condition, moving him or her to the operating room in time to minimize ischemic organ injury. The main stages of voluntary death with subsequent organ donation in the hospital setting are: 1. Receiving a request for MAiD, 2. Processing and confirmation of the request, 3. Discussing the possibility of organ donation at length, 4. Obtaining informed voluntary consent, 5. Conducting the necessary research to confirm the possibility of donation, 6. Planning the procedure for organ donation, 7. Determining the date of the procedure, 8. Hospitalization, 9. Re-affirmation of consent for voluntary death, 10. Actual MAiD, 11. Confirmation of death, 12. Removal of organs [34].

An alternative and, according to the authors, a more humane algorithm for ODE, is the initiation of the procedure of dying at home, in a familiar environment, among relatives. This provides additional comfort and supports the autonomy of the patient's personality. In this case, after receiving a request for MAiD, a special committee is sent to the patient's home, whose members talk to the patient and his relatives about the prospect of organ donation, provide, if necessary, information materials about the euthanasia procedure and the subsequent removal of donor organs, answer questions and express their willingness to provide the necessary support at all stages of the procedure. After receiving voluntary informed consent, the date of the procedure is determined. On the appointed day, the doctor puts the patient into medically induced sleep, conducts physical monitoring for the absence of reaction to external stimuli, then, at home, endotracheal anesthesia is performed, and only after that the future donor is transferred to the hospital, where, without regaining consciousness, he dies with the assistance of doctors and, after death is confirmed, becomes an organ donor [25]. In some cases, the patient's death may occur at home, in which case tracheal intubation is performed after death is confirmed and the deceased is then transported to the hospital for organ donation.

In countries where ODE is possible, the desire to voluntarily pass away is now inextricably linked to the need to decide to carry out organ donation. It is specifically stated that it is not inappropriate to talk to the patient and/or relatives about donation before a final decision

on voluntary death has been made. At the same time, emphasis is placed on the correct coverage of both the practice of voluntary death and the prospect of ODE in the mass media since the success of the procedure depends on its perception by the public and awareness of its citizens [34]. It is emphasized that consent to ODE is a dignified and noble expression of the "last will" of the person who has decided to voluntarily leave life, which has an altruistic basis, correlates with the individual autonomy, is fully consistent with the idea of "death with dignity" and allows "*to give the gift of life to those who need it*" [35].

The legitimization of organ donation after euthanasia as a well-established and ethically acceptable practice has laid the foundation for a new, bolder concept – euthanasia following organ donation. This approach is actively debated as "the optimal way to preserve the quality of donor organs" [36, 37]. In this case, the patient dies from organ removal conducted in compliance with almost all the necessary ethical principles: the patient, based on the autonomy of his or her personality, voluntarily agrees to euthanasia by organ donation, acting at the final stage of his or her life for altruistic reasons. One of the fundamental rules of deceased donation remains unresolved: the dead-donor rule, which states that patients must be declared dead before the removal of any vital organs for transplantation.

Solutions, however, are being proposed. In 2021, Gardieu et al. published a contemporary view of the dead-donor rule in the British Journal of Anesthesia. The authors point out the ambiguity of this principle, citing, for example, the regularly changing neurological criteria for death, which have undergone 7 major revisions since the formulation of the Harvard criteria in 1968, and the World Brain Death Project launched in 2019 further confirms the lack of consensus in the professional community [38]. Ultimately, according to D. Gardieu, rules remain rules exactly as long as professionals are willing to follow them.

The American Medical Association Journal of Ethics in 2020 published an article titled 'Reexamining the Flawed Legal Basis of the "Dead Donor Rule" as a Foundation for Organ Donation Policy', which proposes a radical rethinking of it as a flawed way to protect transplant physicians from legal liability and a move toward developing a legal framework for organ donation that would allow organ donation from still-living patients and make "*medically justifiable homicide*" possible, citing the practice of euthanasia as an example of such "*acceptable forms of homicide*" [39]. To support their position, the authors cite the results of a small sociological study conducted in 2015 in the United States, in which participants were asked to answer a hypothetical question about how acceptable they thought it would be for a patient in a coma to die as a result of removal of their organs for transplantation. This practice was considered legally acceptable by 778 out of 1096 respondents [40]. Now it is necessary to wait for the results of similar sociological studies that would consider a hypothetical scenario of euthanasia due to organ donation in patients experiencing not always unbearable suffering, physical or mental, and in some cases feeling dissatisfied with life.

CONCLUSION

Proponents of euthanasia and, among others, the practice of organ donation after voluntary death regard it as a progressive practice based primarily on compassion. At the same time, they are actively criticized by oncologists and palliative care specialists. In their daily practice, these doctors encounter patients whose chances of recovery are slim, so why do they oppose such a humane way of alleviating suffering as voluntary death?

In a critical article published in the Journal of the American Society of Clinical Oncology, Mark A. O'Rourke et al. oppose the legalization of voluntary physician-assisted dying, viewing the practice as perverse and based on the phenomenon of "extreme autonomy," which boils down to the desire of a terminally ill person to control the timing and circumstances of his or her death, with the basis for the demand to end his or her life with the assistance of a physician being not unbearable suffering, but loss of dignity and the inability to engage in "enjoyable activities". The authors refer to the main postulate of palliative care – there is no situation in which nothing can be done, and they consider the practice of medical suicide as fundamentally contrary to the physician's role as professional vocation, who is obliged to use all his or her strength, knowledge and skills to aid the patient, but in no way to kill the patient [41].

In a publication entitled "*Assisted suicide a 20th century problem, Palliative care a 21st century solution*", palliative care physician, Matthew Dore, also strongly criticizes euthanasia, calling it a regressive practice that has nothing to do with "dignity" and "compassion". Here is a short quote from his work, "*Dr Matt, you know, my dad taught me how to use a spoon, ride a bike, wash and dress, to be fair and generous. He taught me how to be a good husband and father, he taught me how to graciously age… he taught me everything I know, and, you know what, he has now taught me how to die as well.*" [42] In this somewhat naïve quote, the true nature of euthanasia is guessed; the recourse to it competes with the natural course of life, deprives it of fulfillment, of finality, when, through tragic experience, we ultimately realize that dying and death are intimate and integral parts of life. Medical suicide appears in this sense as a surrogate way of "dignified" exit from life, creates an unfounded fear of the dying process and deprives us of the understanding that the only way to cope with this fear is to preempt it.

When analyzing the scientific literature on the problem of euthanasia and organ donation after euthanasia, the ambiguous nature of practices established in Europe and the United States becomes evident. There is a degra-

dation of the institute of bioethics, which has turned from a moral guardian of medicine in several countries into an "ethical screen" covering the exploitation of vulnerable individuals and forcing them, in fact, to die ("with dignity") under the noble pretext of saving someone else's life. Individual autonomy, altruism, personal dignity and the principle of beneficence are purposefully transformed from the fundamental principles of bioethics into surrogate ethical norms that justify "medical murder" in order to solve the organ shortage crisis.

Back in 2012, Australian philosopher and bioethicist Julian Savulescu and Dominic Wilkinson, Professor of Medical Ethics at the University of Oxford, in their article "Should we allow organ donation euthanasia? Alternatives for maximizing the number and quality of organs for transplantation" urged the professional community to follow the ethical principle of maximum utility in order to "maximize the number of organs for transplantation" [43]. It must be stated that the principle of utility is strictly observed, but, unfortunately, it is not mentioned in publications about the noble "dying with dignity" of not always hopeless patients. The decision to end a person's life today, with subsequent organ donation or as a result of organ donation, is increasingly delegated to medical specialists, who rely on a detailed legal framework [35] and therefore have no doubts about the ethical justification of their actions, forcing already vulnerable people to die through the temptation of being useful at least in death.

In Russia, euthanasia is prohibited by law and is not recognized by the Russian Orthodox Church [44]. Criticism of euthanasia and organ donation after euthanasia is necessary as additional confirmation of the correctness of the chosen educational policy in our country. Dialogue with society is focused on the basic principles of bioethics, which form the basis of the cultural code of our society. It is aimed at building public consensus in an atmosphere of trust in such a sensitive issue as organ donation and transplantation.

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LEGAL REGULATION OF BONE BANKS IN THE RUSSIAN FEDERATION

I.A. Kirilova

Novosibirsk Research Institute of Traumatology and Orthopedics, Novosibirsk, Russian Federation

Autologous bone grafts are considered the gold standard for bone grafting, but in cases where their use is limited or impossible, allogeneic bone tissues become the first alternative. To date, the legal status of the activity on manufacturing bone grafts from femoral heads after total hip replacement surgery has not been defined. This somewhat hampers the development of this technology in Russia. Specialized institutions typically use internal instructions developed and approved taking into account existing legal norms in various fields. The creation of uniform operating rules, standards, instructions, clinical guidelines for working with donor tissues, as well as relevant sufficient regulatory and legal support would promote the development of tissue transplantology, bioimplantology and tissue engineering. This, in turn, would open up wide opportunities for the development of new methods of treatment of diseases, injuries, traumas and their consequences.

Keywords: tissue, medical activity, regulations, allogeneic bone tissue, femoral head, procedure legal status.

According to the Russian transplantation law, transplantation of human organs and (or) tissues is a means of saving life and *restoring the health of a person* and should be done in compliance with the laws of the Russian Federation and human rights in accordance with humane principles proclaimed by the international community, *while the interests of the individual must prevail over the interests of society or science* [1].

Proceeding from this, Article 1 of the said law provides that *transplantation* of organs and (or) *tissues from a living or deceased donor is applied* only *if other medical means cannot guarantee* preservation of the patient's (recipient's) life or *restoration of his/her health*; removal of organs and (or) tissues from a living donor is admissible only if his/her health will not be significantly harmed based on a report from a medical council and can take place only with the consent of the living donor; human organs and (or) tissues cannot be sold or purchased; the sale and purchase of such organs and (or) tissues, as well as advertising of these actions is a criminal offense under the laws of the Russian Federation.

To confirm the relevance of this issue, let us consider clinical examples from the specialized scientific literature presented by Majoor et al. [2].

Clinical example #1

A 17-year-old patient. DS: fibrous dysplasia of the left proximal femur. ***Surgical treatment:*** *resection of the mass, plasty with two* ***cortical allografts*** *from the fibula (Fig. 1).*

The patient was removed from further follow-up with good function and no pain [2].

Clinical example #2

A 15-year-old patient. DS: fibrous dysplasia of the right femoral neck (Fig. 2).

Surgical treatment: *resection of the mass, plasty with a* ***cortical autograft*** *from the tibia [2].*

In clinical cases #1 and #2, an alternative to the gold standard (i.e. optimal set of characteristics) was used for bone grafting and restoration of patient's health – cortical allograft from the fibula, an allobone, an osteoplastic material.

Clinical examples are summarized in one publication. Even though the surgical treatment was performed in compliance with similar approaches and surgical technique, a directly opposite result was obtained under a control period. In case #1, treatment was effective and bone graft remodeling was determined radiologically, while in case #2, there were allograft resorption and disease recurrence in the same localization [2].

Clinical examples were given by authors from the Netherlands, where legal issues on allogeneic bone use have been addressed. Is it possible to use allogeneic bone on the territory of the Russian Federation? And can this be done in a way to comply with all regulations in Russia?

The concept of "medical activity" stipulates that providing medical care, conducting medical and physical examinations, sanitary and anti-epidemic (preventive) measures and professional activity related to transplantation of organs and (or) tissues, circulation of donor blood and (or) its components for medical purposes constitute a professional activity (Article 2 of Federal Law No. 323-FZ) [3].

Corresponding author: Irina Kirilova. Address: 17, Frunze str., Novosibirsk, 630091, Russian Federation.
Phone: (913) 958-09-61. E-mail: ikirilova@niito.ru; IrinaKirilova71@mail.ru

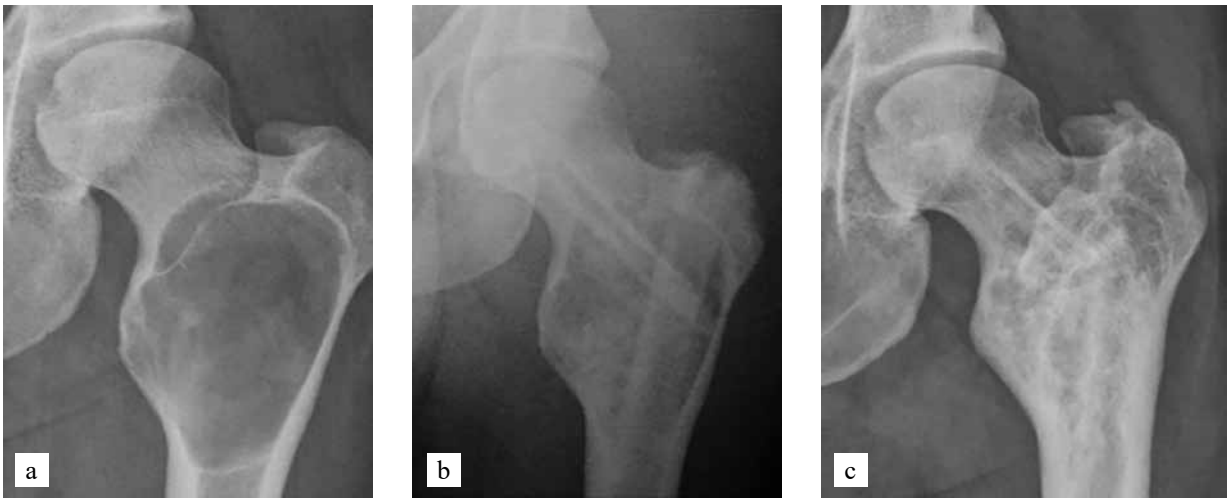
According to Article 12 of the Federal Law No. 99-FZ of May 4, 2011 "On Licensing of Certain Types of Activities", medical activity is subject to licensing [4].

Thus, for a state/municipal institution to be allowed to provide medical care for organ and (or) tissue transplantation, to carry out medical activities related to organ and (or) tissue donation for transplantation, that institution must meet the following requirements:

– obtain a license for the relevant work (services);
– be included in the lists of healthcare institutions that harvest, prepare and transplant human organs and (or) tissues, which are approved by the Russian Ministry of Health (RMH) jointly with the Russian Academy of Medical Sciences (RAMS) [5].

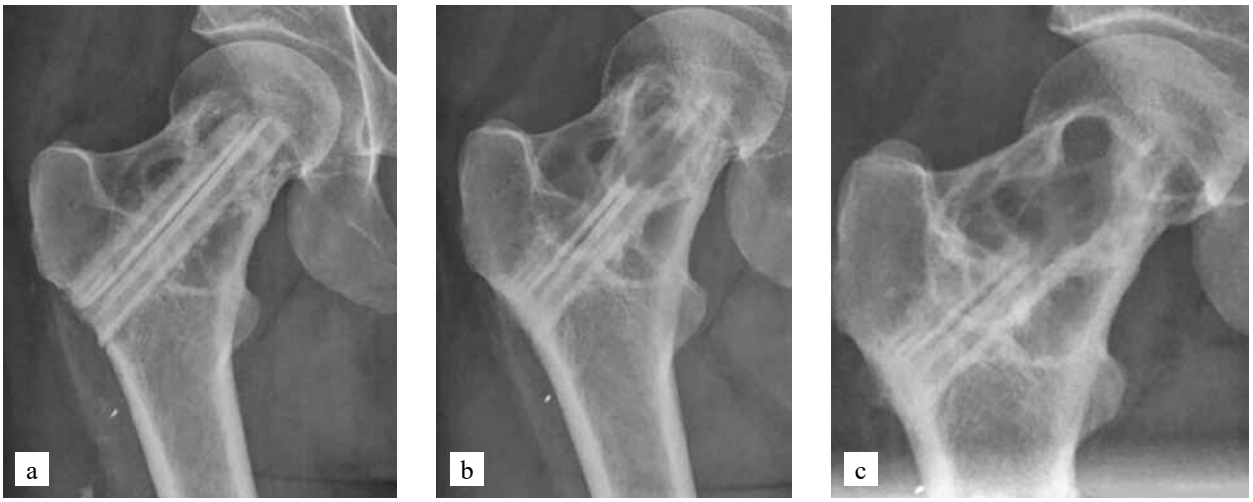
In addition, one of the main requirements for obtaining a license for medical activity is that the medical institution must comply with the Healthcare Procedure, approved by the authorized federal executive body and mandatory for all medical institutions in the territory of the Russian Federation (Article 37 of Federal Law No. 323-FZ) [3].

If we apply the transplant laws of the Russian Federation in full to the activity of manufacturing bone grafts from femoral heads after total hip replacement surgery, then it is necessary to license medical institu-



Preoperative **Control at 3 months** **Control at 7 years**

Fig. 1. Radiographs of a patient with an expansive lesion in the proximal femur with a ground glass aspect and cortical thinning: a, preoperative condition. the diagnosis of fibrous dysplasia was histologically confirmed, and the patient was treated with implantation of two fibular strut grafts; b, control at 3 months: strut grafts gradually incorporated in vital bone with intact (undamaged) bone; c, 7 years after surgery: radiologically positive dynamics with the formation of bone architectonics in the area of surgery [2]



Postoperative **Control at 12 months** **Control at 7 years**

Fig. 2. Dynamic radiographs of the right femoral neck: a, the radiograph is made postoperatively and shows two fibular strut grafts that cross the dysplastic lesion but have minimal contact with vital bone proximally; b, after 1 year, graft resorption gradually increases; c, after 7 years, strut graft is resorbed over the full length of the diameter, losing its stabilizing function

tions for work (services) on procurement and storage of human organs and tissues for transplantation (Table 1). Moreover, the licensing requirements for preparation of removed femoral heads are not defined in Transplantation Procedure No. 567n. In addition, inclusion of medical institutions in Lists No. 738n/3 is required. In this case, contradictions have long been eliminated, as all institutes and centers of traumatology and orthopedics are present in the lists of organizations that perform both procurement and transplantation of organs and (or) tissues.

While these changes are implemented to some extent in multidisciplinary hospitals, it is a problem for single-profile hospitals and federal centers, because it requires large resource costs, whose feasibility is not obvious (Tables 1, 2).

If we apply to these activities Federal Law No. 323-FZ and medical waste regulations, then it is not required to license medical institutions for works (services) on procurement and storage of human organs and tissues for transplantation and include them in Lists No. 738n/3. The activity will be carried out within the framework of a license for work (services) on "traumatology and orthopedics".

In the Russian Federation, medical care in surgery (transplantation of human organs and (or) tissues) and medical activities for removal and storage of human organs and (or) tissues for transplantation may be carried out by state and municipal medical institutions that have been licensed for these types of medical activities in accordance with the established procedure.

In addition, in accordance with the Law No. 4180-I of the Russian Federation "*On Transplantation of Human Organs and (or) Tissues*" of December 22, 1992 (Article 4), such medical institutions should be included in the List of health care institutions engaged in collection, procurement and transplantation of human organs and (or) tissues, approved by RMH and RAMS [5–7]. At the same time, the procedure for their inclusion and exclusion in the List is not defined in the laws of the Russian Federation. This is why medical institutions that decide to run a donor and (or) transplantation program face a well-known legal conflict: in what order should they be licensed and included in the List? The new List of healthcare institutions was approved by RMH and RAMS on November 10, 2022 (order No. 738n/3). It includes 210 medical institutions that harvest and prepare human organs and (or) tissues and 106 medical institutions engaged in transplantation of human organs and (or) tissues.

In accordance with Article 2 of the Law of the Russian Federation "*On Transplantation of Human Organs and (or) Tissues*", RMH and RAMS jointly determine the List of human organs and (or) tissues – objects of transplantation. The current List was approved on June

Table 1

Challenges with the licensing of works (services)

License for transplantology Full implementation of the requirements of the current Regulation (Order No. 567n of the Ministry of Health of the Russian Federation, dated December 21, 2012	**Changing the staffing and organizational structure of the institution** Creation of new units: – Transplantation Department; – Hemodialysis Department; – Pathologoanatomic Department; – Therapeutic Departments; – Organ Donation Coordination Department; – Clinical Immunology Laboratory
	Additional equipment: – Angiography system – Hemodialysis and Hemofiltration Machine – Mass Spectrometer
	Training of highly specialized doctors (orthopedic traumatologists, ophthalmologists, cardiac surgeons) under the following additional specialties: – Surgery – Organ and (or) tissue transplantation

Table 2

Challenges with the licensing of works (services) depending on type of donation

Donation type	
Living	**Deceased**
• Licensing requirements on procurement of removed femoral heads are **not defined** (transplant). • Licensing for type of medical activity is **not required** (medical waste)	• Licensing for this type of medical activity lies under the Office of the Chief Medical Examiner • Licensing for this type of medical activity of the institution lies under the anatomical pathology departments of medical institutions (if the institution has no such department)

4, 2015 via order No. 306n/3 [8], which contains only 25 items (Table 3). Seven items in the List correspond to the types of solid organ transplants that are performed under the state guarantee program for free provision of medical care to citizens (*VMP2*), another 1 is bone marrow and hematopoietic stem cell transplantation (*VMP2*). The remaining 17 items in the List are tissues that can not only be transplanted but also be used for production of medical devices and their application in reconstructive plastic surgery, traumatology and orthopedics, dentistry, and ophthalmology [7, 9].

It is the "tissue part" of the List that raises the most questions among specialists, since the legal status and quantity of tissues may change significantly depending on the technology of their production, processing, registration, and use. Tissues that are not in the List fall out of the legal regulation for clinical use; for example, cartilage, bone, ligaments, dura mater, pericardium, vessels and others.

The wording of paragraphs 4 and 12 – "upper limb and its fragments" and "lower limb and fragments" – is also confusing, since transplantation of the upper limb as a tissue complex was hardly what was meant here. Most likely, this refers to fragments of various tissues topographically located in a given skeletal segment. However, lack of clarifications in the regulatory documentation is a limitation to systematic activities in this direction.

One of the approaches to solving this problem can be to enlarge the positions of the List taking into account global practice, classifiers of human organs and tissues for transplantation existing in other countries. The List of transplant objects, compiled taking into account the classification of the European Committee on Organ Transplantation (CD-P-TO), is presented in Table 4 [9].

In accordance with Article 37 of Federal Law No. 323-FZ of November 21, 2011 "*On the Fundamentals of Public Health Care in the Russian Federation*", health care is organized and provided:

Table 3

List of human organs and (or) tissues – objects of transplantation

No.	Organs (tissues)
1.	Amniotic membrane
2.	Tunica albuginea
3.	Vascularized soft tissue complex, including skin layer, fatty tissue and muscles
4.	Upper limb and its fragments
5.	Temporal fascia
6.	Eyeball (cornea, sclera, lens, retina, conjunctiva)
7.	Intestine and its fragments
8.	Cardiopulmonary complex
9.	Cranial vault bones
10.	Bone marrow and hematopoietic stem cells
11.	Lungs
12.	Lower limb and fragments
13.	Lower jaw
14.	Liver
15.	Pancreas with duodenum
16.	Subcutaneous fatty tissue of the plantar region
17.	Kidneys
18.	Spleen
19.	Heart
20.	Serous capsule of the liver
21.	Vessels (sections of the vascular bed)
22.	Trachea
23.	Renal capsule
24.	Endocrine glands (pituitary, adrenal, thyroid, parathyroid, salivary gland, testis)
25.	Cells intended to replace (perform) their inherent functions in the body, which are obtained (prepared) from biological material as a result of its grinding, homogenization, enzymatic treatment, removal of undesirable components, selective selection of cells and (or) their treatment to remove preservatives in case of their storage and which do not contain other substances (objects), except for water, crystalloids, sterilizing, preservatives, as well as biological material for their preparation

Table 4

List of human organs and (or) tissues according to the CD-P-TO Registry, published annually in the Newsletter Transplant Journal [9]

No.	Organs
1.	Kidney
2.	Liver
3.	Heart
4.	Lung
5.	Cardiopulmonary complex
6.	Pancreas
7.	Pancreas with duodenum
8.	Intestine and its fragments
	Tissues
9.	Bones
10.	Ligaments
11.	Tendons
12.	Fascia
13.	Cartilage
14.	Skeletal muscles
15.	Eye tissues (cornea, sclera, lens, retina, conjunctiva)
16.	Skin
17.	Vessels
18.	Heart valves
19.	Amniotic membrane
20.	Placenta
21.	Bone marrow and hematopoietic stem cells
22.	Adipose tissue
23.	Pancreatic tissue
24.	Endocrine glands
25.	Trachea
26.	Unclassified tissues

– in accordance with the regulations for providing health care by types of health care;
– in accordance with the Healthcare Procedure;
– based on clinical guidelines;
– considering healthcare standards.

Healthcare for organ transplantation, medical activity related to organ and (or) tissue donation for transplantation is regulated by the Healthcare Procedure in the "*Surgery (Transplantation of Human Organs and (or) Tissues)*" Profile, approved by RMH on October 31, 2012 (order No. 567n) (hereinafter referred to as "Healthcare Procedure" or " Procedure") [10]. This Procedure needs to be updated.

The following phrase appears in a piece published in a specialized scientific journal in 2022 [11]:

"The action of the Procedure does not apply to:

– Health care for transplantation of eyeball fragments (cornea, sclera, lens, retina, conjunctiva), which is provided in accordance with the Healthcare Procedure in the "*Ophthalmology*" profile in medical institutions licensed to carry out medical activities, including works (services) in ophthalmology;
– Health care for transplantation of musculoskeletal fragments (bones, cartilage, ligaments, fascia, tendons, muscles, skin), which is provided in accordance with the Healthcare Procedure in the "*Traumatology and Orthopedics*" profile, in medical institutions licensed to carry out medical activities, including works (services) in traumatology and orthopedics [12];
– Health care with the use of medical products that were prepared using tissue components;
– Medical activities related to the procurement of human cadaveric tissues at the thanatology departments of forensic medical examination bureaus and pathology departments of medical institutions;
– Medical activities related to the procurement of human cadaveric tissues for preparation of medical products" [11].

However, there are no references or interpretations in the regulatory documents (Fig. 3), which again can be interpreted at present only as the opinion of the head of a specialized department at RMH and the RMH chief freelance specialist for that field.

Thus, at present there are certain "scissors" in the laws of the Russian Federation, consisting of the following:

Health care should be provided in accordance with the standards and procedures for provision of health care, i.e. with registered medical products, and the registration of allogenic products is not possible because the source of "raw materials" and the legitimacy of obtaining allogenic bone tissue and its use have not been fully addressed.

According to Sergey Gautier, Fellow, Russian Academy of Sciences, chief freelance transplant specialist at RMH, the key issue is actualization of the regulatory and legal framework in the field of donation and transplantation of human organs and tissues *at the level of federal subjects of the Russian Federation*.

As an example of a systemic regulatory and legal act in the field of organ donation and transplantation at

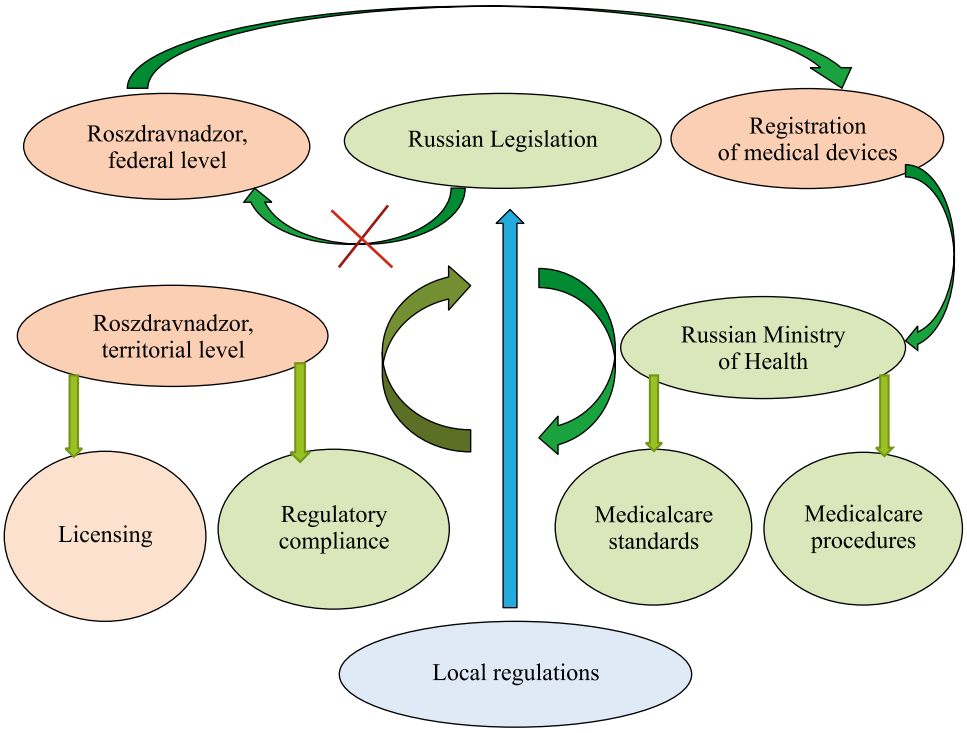


Fig. 3. Tissue bank regulatory challenges. Roszdravnadzor, Federal Service for Surveillance in Healthcare

the level of federal subjects is an Order (No. 737) of the Department of Health of Moscow of October 19, 2017 "On Medical Activities Related to Donation of Human Organs and the Provision of Medical Care in the "Surgery" (transplantation of human organs and tissues) profile in the city of Moscow". However, despite its title, this document is devoted exclusively to organizational issues of organ donation, contains gaps in the part of tissues and could be transferred to the level of a federal subject of the Russian Federation.

It is recommended to transfer a number of issues subject to legal regulation to the level of a federal subject of the Russian Federation, for example:

1. Procedure for implementation of medical activities related to tissue donation for the purpose of tissue transplantation and (or) manufacturing of medical devices.
2. Procedure for interaction with the forensic medical examination bureau when conducting activities related to organ and (or) tissue donation for the purpose of transplantation.
3. Procedure for maintaining a waiting list and distributing donor tissues for transplantation and (or) manufacturing of medical products.
4. Regulations on regional coordination centers of organ and (or) tissue donation.
5. Regulations on interaction with medical institutions carrying out activities in the field of organ and (or) tissue donation and (or) transplantation on the territory of other federal subjects of the Russian Federation.
6. Registers of medical institutions carrying out medical activities related to organ and (or) tissue donation and (or) transplantation.
7. Standard forms of documentation in implementation of medical activities on organ and (or) tissue donation and (or) transplantation [13].

We would like to note that this statement is in contradiction with Federal Law FZ-323, which states that *all citizens of the Russian Federation have the right to quality healthcare regardless of their place of residence*. Transferring the regulatory framework to the regional level allows for regional differences in such an important area of activity and makes the need for regulatory and legal regulation vital for the industry as a whole, and for specialists and patients in particular.

Back in 2016, we analyzed the legislation of the Russian Federation regarding the legal norms regulating tissue donation and transplantation; we also analyzed the appeals received by the chief freelance transplant specialist at RMH on this issue.

Based on the study, it was concluded that "*gaps and conflicts in the legal regulation of tissue donation and transplantation in the Russian Federation exist, are significant and should be eliminated. This is one of the basic conditions for further development of tissue transplantology in the Russian Federation*" [14].

The following tasks were formulated as priority tasks for improving the legal framework:

1. Remove the legal conflict "licensing – lists" in Decree No. 291 of the Government of the Russian Federation of April 16, 2012 (see item 4, sub-item 3, paragraph 2).
2. Clarify the list of licensed works and services in the "*Transplantation*" profile in accordance with the object (type) of transplantation. Study the issue of state supervision over production and circulation of medical products using human tissues (part 5 of Article 38 of Federal Law 323-FZ).
3. Develop and approve the Regulation on procurement of donor tissues, including requirements for the standard of equipment and staffing of specialists of medical institutions to carry out this activity. Clarify the legal regime for the procurement of donor tissues (Article 47 or Article 68 of Federal Law 323-FZ).
4. Develop and approve Regulations on the activity of tissue banks, make appropriate changes to the Healthcare Procedure in the "Ophthalmology" (eye banks) profile, to the Healthcare Procedure in the "traumatology and orthopedics" (bone banks) profile.
5. Clarify the legal status of the activity on manufacturing bone grafts from femoral heads after total hip replacement surgery.
6. Develop and approve separate accounting and reporting forms for human tissue donation and transplantation, make appropriate changes to Order No. 355n of the Russian Ministry of Health.
7. Develop methodological guidelines on procurement of donor tissues and on the operation of tissue banks.
8. Supplement the state program of the Russian Federation "Health Care Development" with measures on tissue transplantology, including the activity of forensic medical examination bureaus on procurement of donor tissues and the activity of tissue banks.

These tasks remain relevant today.

Till now, the legal regulation is undergoing changes, but, unfortunately, it concerns only organ donation. At the same time, the word "tissues" always appears in the title of regulatory documents. That is why the release of each Order or supplement to Orders of the Russian Ministry of Health arouses interest among specialists dealing exclusively with the issues of tissue procurement and use within the framework of separate surgical specialties.

A vivid example is such a direction as "Traumatology and Orthopedics".

In the Russian Federation, 30,000–35,000 total hip arthroplasty operations are performed annually, and the need, according to expert estimates, is at least 100,000 operations per year [15]. After such surgeries, a valuable biomaterial – removed femoral heads – remains. After several manipulations, it can be used in reconstructive and plastic surgeries to replace bone tissue defects.

To date, the legal status of the activity on production of bone grafts from femoral heads after total hip arthroplasty has not been defined. This somewhat complicates development of this technology in the country.

The entire donor tissue workflow can be divided into three main steps [16, 17]:

– Donation;
– Storage, processing and sterilization;
– Clinical application.

It should be remembered that human tissue is a unique biological structure. By its structure and functional properties, any tissue is unique, and in cases of massive lesions or pathological changes, it can be irreparable using its own regenerative resources.

Autologous tissues for transplantation are considered the gold standard, but in cases where their use is limited or impossible, allogeneic tissues become the first alternative.

In contrast to organ transplantation, where the main principle is preservation of organ viability, the preservation of their biological activity and structure (after processing and sterilization), which ultimately leads to positive treatment outcome, is of crucial importance in allogeneic tissue transplantation [18, 19].

In world clinical practice, the femoral head is singled out as a separate unit among donor tissues of living donors. A resected femoral head is an available donor material suitable for further use both as a native (fresh frozen) graft and as other biomaterials made from it, different in shape, processing method and sterilization.

On the territory of the Russian Federation, an organizational and functional model of femoral head procurement with the compilation of process flowcharts and provision of mandatory documentation was proposed in 2009 [20]. For the first time in national literature, a system based on the process approach is presented, covering organizational, ethical and technical issues, taking into account the existing regulatory and legal framework. Development of this process made it possible to preserve valuable bioplastic material necessary not only for revision interventions on large joints, but also for other pathologies associated with bone tissue deficiency in a separate institution.

The above-mentioned phasing of donor tissue processes is given for a better understanding of the fact that different phases are currently under the responsibility of different professional communities and legal regulations [17, 19].

Under current legislation, tissue extraction is permitted for scientific, educational and therapeutic purposes; these purposes are different in nature. Working with donor tissues requires government regulation.

According to the chief transplant specialist at RMH, *it is inappropriate to apply the human organ/tissue transplantation laws of the Russian Federation* to this biomaterial since in this case, there are no operations involving tissue harvesting from the donor and tissue transplantation to the recipient. The material is obtained as a result of a hip replacement surgery performed on a patient for medical indications and is used after deep processing as a medical product in several patients when performing reconstructive plastic surgeries to replace bone tissue defects. In essence, this biomaterial is a medical waste, since, according to Russian law, medical waste includes anatomical waste generated in the course of medical activities. Unfortunately, Federal Law 323-FZ in Article 49 does not provide for the possibility (does not establish rules) of processing anatomical medical waste into medical products [21].

On this issue, we agree with Sergey Gautier and believe that the legislation on transplantation approaches the direction only selectively. Any biomaterial, taken even during surgery, before undergoing technological processing, must be diagnosed to ensure there is no risk of transmitting bloodborne infections. Specialists have no alternative method of diagnostics other than additional examination of the patient as a tissue donor. It is this tissue procurement stage that falls into the zone of regulation in accordance with the "*Transplantology*" profile, since in the preoperative period, the patient's blood is collected for PCR (polymerase chain reaction) and ELISA (enzyme-linked immunosorbent assay) diagnostics of infectious processes. Even though hospitalization is scheduled and compulsory examinations, including for bloodborne infections, have already been conducted, infections are detected in 10–15% of cases even at this stage of additional examination of the patient as a donor.

For patients from whom femoral heads removed after prosthetics remain, the rules on informed voluntary consent (Article 20 of Federal Law 323-FZ) to use their biomaterial for medical products should be applied. Regarding the production and use of bone grafts, the rules of Article 38 of Federal Law 323-FZ on medical products apply [21].

At present, there are no uniform guidelines in Russia that would regulate the handling of this donor material, there is no clear definition classifying this biomaterial, and there is no objective information on the number of primary hip replacement surgeries and the number of resected heads and their further use. Nevertheless, attempts are constantly being made to standardize technological processes by different institutions at their bases.

Proposals for standardization of Russian tissue banks were prepared based on the concept of mass allotissue procurement in any conditions, not only in the aseptic conditions of the operating room, which was proposed by Vladimir Savelyev, the head (1962–1973) of the laboratory of biotissue procurement and preservation at Novosibirsk Research Institute of Traumatology and Orthopedics. However, further development of the standardization process with the formation of regulatory legal

acts throughout the Russian Federation, unfortunately, did not happen [20, 22, 23].

Specialized institutions, as a rule, use internal instructions developed and approved taking into account the existing legal norms.

Emerging publications [17–20, 24] and methodological guidelines [25] can serve as a confirmation. Methodical guidelines "*Safety and Quality Control of Allogeneic Human Tissue Transplants*" of the Moscow City Department, dated 2022, agreed upon by the chief freelance transplant specialist at Moscow City Health Department, fully describe the processes and approaches used in the tissue banks of the Russian Federation. These guidelines are based on various regulatory documents that have not yet been formed into a coherent system but ensure maximum safety and efficiency. At the same time, some of the documents relate to legislation on donors, and some to the regulatory documents on medical products. But, again, everyone should realize that it is not possible to use regulations concerning medical products in isolation. This is because "raw materials" here are not metals and plastics that can be standardized, but are human tissue fragments, i.e. standardization can only concern the criteria for donor selection, infectious safety, structural integrity, processing technology, including final sterilization and bacteriological control.

Currently in the Russian Federation, tissue grafts are outside the legal field, which dramatically complicates both the process of their production and their use in clinical practice [24].

Thus, working with donor tissues implies complex interprofessional interaction, all stages of which should be regulated [17, 19]. Creation of unified rules, standards, instructions, clinical guidelines for working with donor tissues, as well as having sufficient regulatory and legal support will contribute to the development of tissue transplantology, bioimplantology and tissue engineering, which, in turn, will open up wide opportunities for the development of new methods for the treatment of diseases, injuries, traumas and their consequences [25].

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LUNG DONATION AFTER CARDIAC ARREST. CHALLENGES AND OPPORTUNITIES. LITERATURE REVIEW

I.V. Pashkov[1], M.G. Minina[1, 2], N.V. Grudinin[1], V.K. Bogdanov[1]

[1] Shumakov National Medical Research Center of Transplantology and Artificial Organs, Moscow, Russian Federation

[2] Botkin Hospital, Moscow, Russian Federation

The global development of transplantology faces several objective obstacles. One of the major ones is widespread organ shortage. This is most pronounced in clinical lung transplantation (LT). The development of this area is directly connected with more intensive development of available donor resources and search for new sources of donor organs that are suitable for transplantation. Along with the existing methods of increasing the number of lungs suitable for transplantation, LT with donation after cardiac death (DCD) is attracting increasing attention. The effectiveness of this approach has been confirmed by the International Society for Heart and Lung Transplantation and deserves more attention from Russian specialists.

Keywords: lung transplantation, lung donation, effective circulatory arrest, brain death, cardiac arrest, hypoxic necrobiosis.

LT is the only effective way to cure terminal respiratory failure against the background of refractory chronic lung diseases of various etiologies.

In Russia, LT can be characterized as a relatively young field. The number of transplants performed (national experience) is much lower than in Europe and North America in contrast to the transplants of other solid organs. As of January 2024, 207 lung transplants have been performed in the Russian Federation, including 17 transplants of heart-lung complexes.

Finding ways to solve the problem of shortage of donor lungs suitable for transplantation is a priority task for further development of this area of clinical transplantology.

Accumulated international experience demonstrates several promising areas for improving the quantitative and qualitative indicators of LT associated with improved surgical approaches to operations on recipients. It is possible to use the available donor resource more intensively by performing two single-lung transplants instead of one double-lung, the outcomes of which, according to some authors, are comparable [1]; performing lobar LT [2], split LT for patients with small anthropometric parameters [3], transplantation of two lung lobes from 2 living donors [4–6].

Another way involves expanding the criteria of organ donors for transplantation without compromising transplant outcomes [7, 8], namely, the use of lung recruitment methods within the framework of multi-organ donor conditioning [9, 10]; the use of lungs of a suboptimal donor followed by normothermic extracorporeal lung perfusion [11, 12].

The listed options are used mainly in conditions of donation after brain death (hereinafter referred to as "brain-dead donor", BDD) with preserved blood circulation in the donor.

Irreversible injury to human organs and tissues as a result of cardiac arrest occurs due to hypoxia and ischemia. Tolerance of solid organs to the hypoxic effects varies widely. Hypoxia impairs cellular respiration (oxidative phosphorylation) resulting in acute deficiency of macroergic compounds (primarily adenosine triphosphate, ATP) and elevated levels of its metabolites (adenosine diphosphate, ADP; adenosine monophosphate, AMP; etc.). In connection with this, further energy supply is carried out anaerobically. ATP deficiency is replenished by the reaction of anaerobic glycolysis. There is a rapid depletion of glycogen reserves, accumulation of products (metabolites) of glycolytic reactions – lactic and pyruvic acid – which leads to acidification of the intracellular environment and suppression of anaerobic glycolysis. Cellular energy supply completely stops. All cellular energy-dependent reactions stop. Transmembrane transport of potassium and sodium ions against a concentration gradient is impaired. Cellular homeostasis is disrupted. Passive cell membrane permeability leads to increased intracellular sodium and potassium deficiency, impaired repolarization processes, suppression of functional activity, and loss of action potential. Excessive intracellular sodium content leads to cellular hyperhydration. High intracellular concentration of calcium entering through inactive voltage-gated calcium channels activates membrane phospholipases and nuclear endonucleases. The totality of the occurring processes, a cascade of bioche-

Corresponding author: Ivan Pashkov. Address: 1, Shchukinskaya str., Moscow, 123182, Russian Federation. Phone: (905)521-36-67. E-mail: dr.pashkov.ivan@gmail.com

mical reactions, leads to damage of cell membranes and its structural elements up to cell death [13, 14].

Thus, the mechanism described above limits the acceptable time frame for obtaining a viable donor organ, and in some cases makes it basically impossible.

In this context, lungs have an undeniable advantage over other solid organs. Under certain conditions, the lungs can resist warm ischemia effects, because lung parenchyma cells are initially adapted to absorb oxygen from alveolar gas, and, therefore, need less oxygenation by perfusion. Thus, in relation to the lungs, warm ischemia is not identical to tissue hypoxia.

This hypothesis has been confirmed in several experimental studies.

Using a dog model, Egan et al. demonstrated the principal possibility of transplanting lungs procured within 4 hours after circulatory arrest. The experimental design consisted of left LT from donors 1, 2, 4 hours after cardiac arrest. One hour after transplantation, the pulmonary artery and right main bronchus were ligated, after which respiratory function and gas exchange were performed exclusively by the single transplanted lung. The best survival and gas exchange rates were obtained in the group with the shortest warm ischemic time. All recipients of 1-hour cadaver lungs safely survived the 8-hour follow-up period with satisfactory gas exchange rates. Two of 5 animals in group 2 (2-hour cadaver) showed similar outcomes. In group 3, gas exchange and survival rates were unsatisfactory [15].

Two years later, Ulicny Jr et al. supplemented the design of the Egan et al. experiment with postmortem lung ventilation during a 4-hour warm ischemic period, which, all other things being equal, resulted in a 100% 8-hour survival rate, whereas in the non-ventilated group, survival rates were significantly lower [16].

In a series of experiments, D'Armini et al. evaluated and compared the number of viable lung cells of laboratory rats and their metabolic activity depending on the use of artificial ventilation. The number of non-viable lung cells at 2, 4, and 12 hours after circulatory arrest and in the absence of artificial ventilation amounted to 36%, 52%, 77% respectively, while postmortem ventilation achieved significantly better values: 13%, 10%, 26% at similar control points ($p < 0.01$). Evaluation of the levels of ATP and its metabolites in the experimental groups showed that in the case of lung ventilation with oxygen, the process of aerobic oxidation and oxidative phosphorylation is preserved (comparatively higher level of ATP at control points), i.e. to preservation of metabolic activity, hence viability is preserved [17].

Thus, postmortem lung ventilation in the experiment made it possible to preserve the viability and functional activity of lung parenchyma cells (if not avoided, but significantly reduced the intensity of cell death). These results can be achieved only during artificial ventilation with high oxygen content in the respiratory mixture [18].

The possibility to maintain lung oxygenation after biological death and cessation of spontaneous breathing in the donor by continuing artificial lung ventilation is an important advantage in comparison with other solid organs in the context of organ transplantation from a donor after circulatory death (DCD).

The listed research results demonstrate the fundamental possibility of performing transplantation of lungs subjected to warm ischemia in the donor's body. The effectiveness of this approach is determined by warm ischemic time. According to some reports, acceptable ischemia periods are up to 4 hours, which allows to consider a donor with circulatory arrest as a lung donor as well.

The concept of lung donation after circulatory arrest has a clear physiologic rationale and has important advantages with respect to lung donation after brain death.

Today, postmortem organ donation from a BDD is the generally accepted gold standard of clinical transplantology. The events leading to the development of this condition are most often acute in nature – direct traumatic impact with destruction of the brain matter, vascular accidents, which have an impact both due to mass effect with dislocation of structures and due to lesion of brain stem structures.

Considering brain death not as an end result but as a process, we can identify a number of regular sequential events, the key to which is development of cerebral edema with subsequent brain stem compression and herniation, which leads to loss of central regulation of the parasympathetic (autonomic) nervous system. These circumstances naturally lead to impairment of systemic hemodynamics, development of systemic inflammatory reactions (catecholamine and cytokine storms), water-electrolyte disorders and other events that have a direct damaging effect on the donor's lungs. The combined effect of the above factors can lead to the so-called "neurogenic pulmonary edema".

The nature and structure of morphological changes in the lungs of patients who died within 12 hours after traumatic brain injury correspond to acute respiratory distress syndrome [19]. The incidence of pulmonary edema associated with damage to brain structures of various etiologies differs depending on the time from development of brain death. According to Rogers et al, pulmonary edema on autopsy of patients who died on the spot as a result of traumatic brain injury was observed in 32% of cases, whereas after 96 hours these changes were observed in 50% of cases [20]. Several factors have been identified as having a damaging effect on the lungs of the patient and donor. They are: increased plasma levels of endogenous catecholamines resulting from sympathetic nervous system activation, which occurs during acute brain injury, and has been called "catecholamine storm"; pulmonary edema due to hypertension in the pulmonary circulation against the background of acute left ventricu-

lar failure; increased permeability of pulmonary capillaries as a response to intracranial hypertension. Systemic inflammatory reaction that has a damaging effect on the endothelium of the pulmonary vascular bed due to circulating proinflammatory cytokines, mediators of systemic inflammatory response, the source of which can be the damaged brain matter, has been called "cytokine storm" [21]. The absence of the above-mentioned damaging factors is an important advantage of lungs from donors after circulatory death.

Donors after circulatory death represent a heterogeneous group of patients, clinical circumstances, timing and types of care. The earliest attempts at systematization date back to 1995, when a classification of donors after circulatory death, named after the place of its adoption, Maastricht, was formulated (Table 1) [22].

In principle, there are 2 classes of donors: patients with uncontrolled (categories I and II) and those with controlled circulatory arrest (categories III and IV). The first category includes patients found without signs of cardiac and respiratory activity, with no known timing of the onset of circulatory arrest or other events (circumstances) leading to it. The second category includes patients found with other comparable circumstances, but with witnesses available to determine the time of onset of circulatory arrest. The third and fourth categories include hospitalized patients for whom cardiac arrest is foreseeable and expected. These are patients whose vital signs can be maintained with ventilator support, including patients with confirmed brain death. Later, the classification was extended to category V – donors after euthanasia.

Category 3 controlled donors are most often used for clinical transplantation. Controlled donors have several advantages, because their stay in the hospital implies the availability of clinically relevant information for the transplantologist, such as infectious status, presence or absence of clinically significant diseases and conditions that influence the decision on organ transplantation. The period of functioning of life support systems can be used to conduct fundamentally important studies that determine the quality of the donor organ. The process of interrupting life support is clearly regulated and allows all necessary technical and organizational measures to be taken in advance to prepare the recipient in order to minimize warm ischemia time. Working with controlled donors is strictly regulated by national laws.

Table 1

Classification of donors after circulatory death (Maastricht, 1995)

Dead on arrival	Category I
Unsuccessful cardiopulmonary resuscitation	Category II
Expected cardiac arrest	Category III
Cardiac arrest with established diagnosis of brain death	Category IV

Within the framework of national clinical transplantology, the existence of category 3 (expected, actually planned cardiac arrest by stopping the functioning of life support systems) is not regulated by the current legislation.

The current legal "window of opportunity" allows for implementation of activities related to the conditioning and removal of organs from donors corresponding to Maastricht I and II categories [23].

In 2011, The International Society for Heart and Lung Transplantation (ISHLT) formed a working group to create a registry of lung transplants from donors after circulatory arrest. The first attempts to estimate the contribution of this lung source to the total number of globally performed lung transplants date back to 2015. A team of authors led by Marcelo Cypel evaluated the current experience between 2003 and 2013. There were 10 transplant centers in North America, Europe and Australia.

The article retrospectively evaluates the efficacy and safety of LT technique on the example of 306 DCD cases in comparison with the classical concept of BDD lung transplantation (totaling 3,992 cases) [24].

Of the 306 DCDs, the vast majority were categorized as Maastricht 3 (94.8%), Maastricht 4 (4%), and Maastricht 5 (euthanasia, 1.2%). It is noteworthy that there is no record of lung transplants from Maastricht category 1 and 2 DCDs in the first registry.

The immediate efficacy of lung donation in the absence of circulation was evaluated by the level of 30-day survival (DCD, 96%; BDD, 97%), 1-year survival (DCD, 89%; BDD, 88%; $p = 0.59$), and 5-year survival, which in both groups was 61%.

Of note is the limited use of ex vivo lung perfusion (EVLP) (only 12%), which most likely reflected the availability of this technique at that time. On the other hand, the overwhelming use of Maastricht category 3 donors in DCD fundamentally allows to obtain high outcomes even without EVLP, because with proper organization, the duration of warm ischemia of DCD lungs can be minimized to a time comparable with BDD. However, the authors suggest that extracorporeal normothermic lung perfusion has great prospects, especially within the framework of Maastricht categories 1 and 2 [24].

The next revision of the registry and its results was in 2019 and covers the period from 2003 to 2017. It demonstrates a positive trend of a twofold increase in the number of transplant centers over a 5-year period (from 11 centers in 2013 to 22 centers in 2017). The registry includes 11,516 lung transplants, of which 1,090 (9.5%) were performed in a DCD setting. The vast majority (94.1%) fell under Maastricht category 3, while categories 1 and 2 featured less than 1% (Fig.).

On the other hand, in the period from 2005 to 2016, Spanish and Italian authors, demonstrating their own

Table 2 Number of DCD Lung Transplants at Participating Hospitals by Category between January 1, 2003 and June 30, 2017

Maastricht category	N	%
(I) Dead on arrival (uncontrolled)	1	0.1
(II) Unsuccessful resuscitation (uncontrolled)	6	0.6
(III) Awaiting cardiac arrest (controlled)	1,026	94.1
(IV) Cardiac arrest in a brain dead donor (controlled)	43	3.9
(V) Euthanasia (controlled)	14	1.3
All	1,090	100.0

Abbreviations: DCD, donation after circulatory death
Adapted from Kootstra et al.[1995] and Detry et al.[2012]

Fig. Lung transplants from donors after effective circulatory arrest depending on the Maastricht category [25]

experience and outcomes of LT from DCD categories M1 and M2, published a number of studies [26–29].

The dynamics of growth of the specific volume of lung transplants from DCD in the period from 2003 (0.6%, 3 out of 530 lung transplants) to 2016 (13.5%, 146 of 1,081 lung transplants) is clear. At individual transplant centers, the number of lung transplants performed from donors after circulatory arrest reaches 28% to 40% [25, 30].

It is noteworthy that only 4 hospitals performed over 100 DCD transplants during this period (2003–2017). The undoubted leaders are Toronto General Hospital (160); Alfred hospital, Australia (148); University Hospital Gasthuisberg Leuven, Belgium (116); Universitair Medisch centrum Groningen, Netherlands (111). The largest number of transplant centers demonstrating their own experience within the framework of ISHLT DCD registry is located in the US (8), the UK (6), and Australia (4). Other clinics are represented in their own countries with one transplant center each.

Still noteworthy is the absence of hospitals and authors specializing in Maastricht categories 1 and 2 patients in the registry, despite a significant number of publications devoted to this topic [31, 32].

The second edition of the DCD registry allowed us to demonstrate the effectiveness of LT from the classical donation after brain death (DBD) and donation after cardiac death (DCD) based on more evidence (Table 2).

Table 2

Comparative outcomes of lung transplantation with donation after cardiac death (DCD) and with donation after brain death (DBD) [25]

	30-day survival	1-year survival	5-year survival
DCD	96%	89%	63%
DBD	97%	88%	61%
p	(p = 0.30)	(p = 0.44)	(p = 0.72)

The present results provide a compelling rationale for the use of donors after circulatory arrest as lung donors, as patient survival rates obtained are comparable to those of LT from brain-dead donors.

CONCLUSION

Accumulated experience of LT from DCD donors within the international community of heart and lung transplantation, demonstration of comparable immediate and long-term outcomes and increased number of such transplant surgeries at leading transplant centers, suggest that this source of donor lungs is a possible and prospective one. Development of this direction in Russia, especially in regions with high donor activity (for example, Moscow) will increase the number of lung transplants performed, thereby increasing the availability of such a complex type of transplantation in the hospital.

The authors declare no conflict of interest.

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STRUCTURAL EVOLUTION OF MECHANICAL HEART VALVES (REVIEW)

M.A. Lepilin[1], A.V. Bogachev-Prokophiev[1], M.O. Zhulkov[1], D.S. Khvan[1], D.A. Sirota[1,3], A.G. Makaev[1], A.V. Protopopov[1], A.S. Grenadyorov[2], Kh.A. Agaeva[1], A.M. Chernyavskiy[1,3]

[1] Meshalkin National Medical Research Center, Novosibirsk, Russian Federation
[2] Institute of High Current Electronics, Tomsk, Russian Federation
[3] Novosibirsk State Medical University, Novosibirsk, Russian Federation

Prosthetic heart valves are widely used biomedical devices. The need for these prostheses is increasing due to the increasing life expectancy of the general population and the consequent incidence of age-related degenerative valvular defects. However, even though mechanical prosthetic valves have been significantly modernized over the last decades, they are still associated with several life-threatening complications, the main one being thrombosis. Addressing this problem is challenging and requires collaboration between bioengineering and cardiothoracic surgery. Thus, the problem of creating the most adapted model of prosthetic heart valve (PHV) turns out to be at the confluence of sciences – medicine, biology, applied mechanics, mathematical modeling, etc. Today, it seems clear that the engineering ideas for hemodynamic adaptation of PHV models have been fully developed. However, research in the field of materials science, as well as a search for surface modification methods, remain a pressing bioengineering challenge.

Keywords: heart valves, mechanical valves, acquired heart diseases.

The prevalence of mitral and/or aortic heart disease is above 10% among patients aged >75 years and it continues to increase every year [1–3]. Prosthetic heart valve replacement remains an effective and often the only possible way to treat heart valve diseases. This procedure eliminates pathologically altered structures, improves intracardiac hemodynamics and patient's quality of life [4, 5]. Since the first aortic valve replacement surgery was performed in March 1960 by Dwight Harken at Boston City Hospital, hundreds of thousands of such interventions have been carried out [6, 7]. However, despite the obvious progress in the development of PHV models and improvement of surgical implantation technique, the postoperative period is associated by a high risk of several complications [8–10]. According to surgical registries, between 250,000 and 280,000 prosthetic heart valves are implanted worldwide each year: the approximate ratio is 50/50 between mechanical and biological ones [5, 11].

Despite the availability of many modern anticoagulants and antiplatelet agents, the use of even the latest PHV models is associated with thromboembolic events in 0.7–6.4% of patients [12]. According to Dangas et al., thromboembolic syndrome occurs in 0.1–5.7% of cases [13]. Studies by Pibarot et al. showed that about 10% of patients with implanted mechanical PHV have one episode of thromboembolism per year [14]. At the same time, the incidence of PHV dysfunction ranges from 0.4% to 6.0% per year of the total number of prosthetic operations performed. According to many authors, this figure is significantly underestimated because routine screening aimed at detecting prosthetic valve dysfunction in the postoperative period is not performed in most cases if there are no clinical symptoms that would lead to suspicion of dysfunction [15–18]. Implantation of mechanical PHVs requires lifelong use of anticoagulants, which is also associated with a risk of complications. Patients taking oral anticoagulants to prevent thromboembolism are prone to hemorrhage, especially retroperitoneal, gastrointestinal, and intracranial hemorrhage. Bleeding complications occur in approximately 4% of patients annually, with 5–10% of these events resulting in death [16]. In addition to anticoagulants, patients at high risk of thromboembolism take platelet inhibitors, which are associated with a 55% increased risk of bleeding [17].

The development of reliable design and the search for inert/hypothrombogenic materials have been the subject of many years of scientific and engineering research. Since the first mechanical PHV models were introduced, they have been continuously improved. However, creating a PHV model that fully matches the characteristics of native human heart valves remains a dream for designers, cardiac surgeons, and patients.

The main function of any PHV is to provide unidirectional blood flow. An ideal prosthesis should meet the following requirements: it should have a reliable and fairly simple design that pressures long-term continuous functioning for decades; it should have good hemodynamic characteristics, i.e. it should provide laminar blood

Corresponding author: Andrey Protopopov. Address: 15, Rechkunovskaya str., Novosibirsk, 630055, Russian Federation. Phone: (914) 708-39-79. E-mail: andrew-uss@yandex.ru

flow that is as close as possible to physiological characteristics, and should not create excessive pressure gradient between the heart chambers that it shares; it should be biologically inert, be hemocompatible/hypothrombogenic, be easy to implant, have good radiographic visibility, and have low noise characteristics [18].

The invention of balloon-expandable prosthetic valves undoubtedly provided a tremendous boost to surgical treatment of heart valve diseases. Since the introduction of the Starr–Edwards PHV model in 1960 through 1998, over 175,000 such valves have been implanted in mitral, aortic, or tricuspid positions [19]. However, the large weight, height (profile), and inertia of the locking element limited the use of balloon models in many cases: in patients with severe mitral stenosis, in patients with a small left ventricular cavity, etc. (Fig. 1).

The Starr–Edwards model underwent 8 modifications between 1960 and 1965 based on surgeon feedback, analysis of postoperative complications, and patient outcomes. The last improved model was then used unchanged until 2004 [20]. The use of ball-locking prosthetic models was often associated with turbulent flow, episodes of thromboembolism, and lack of effective orifice area.

According to Best et al., during the first year after implantation of the Starr–Edwards model, mortality was

Fig. 1. Starr–Edwards mechanical heart valve model

Fig. 2. Björk–Shiley mechanical heart valve model

21%; over the next 7 years, this rate decreased to an average of 3% per year [21]. However, 10-year freedom from prosthetic valve thrombosis, thromboembolism, and prosthetic valve endocarditis for the Starr–Edwards model was 91, 91, and 97, respectively [22–24].

The results described were primarily associated with imperfections in the design of the prosthesis itself. However, in fact, a significant decrease in the incidence of thromboembolic complications after implantation of this PHV model over time was down to the evolution of anticoagulant therapy protocols. By 1997, freedom from this type of complication ranged from 74% to 87% at 10 years after implantation [25, 26]. Nevertheless, in November 2015, Albert Starr documented cases of the longest functioning of the Starr–Edwards balloon model after primary implantation – 51.7 years and 44.4 years in aortic and mitral positions, respectively [20].

The next fundamentally new PHV models were rotary disc valves, where the closing element was a disc that rotates around an eccentric axis, thus opening and closing the flow orifice (Fig. 2).

The hemodynamic characteristics of this model were significantly better than ball valves. The dimensions of the valve allowed safe implantation in cases where the annulus fibrosus was small and avoided the development of low cardiac output syndrome in patients with a "small" left ventricle. The first implantation of the most successful model of disc prosthesis, the Björk–Shiley valve, was performed in Sweden in 1969 [27]. Structurally, this valve was a freely moving disc occluder enclosed in a Teflon-treated stellite cage. The opening angle of the valve was 60 ± 2° [28], nevertheless, this angle was quite sufficient to prevent excessive hemolysis.

So in a study by Falk et al. [29], the serum lactate dehydrogenase (LDH) level was elevated in all patients who had the Starr–Edwards valve implanted in the aortic position, but was elevated only in one third of patients after implantation of the Björk–Shiley model. The size of the prosthesis was of particular importance, as smaller balloon valves especially in the aortic position caused more significant hemolysis than larger prosthetic valves. However, the degree of hemolysis in the case of the Björk–Shiley model is so small that the size of the prosthesis had little or no effect. According to Björk, in a group of 1657 patients carrying Björk–Shiley valve models, the 15-year actuarial survival rate was 54%, and thromboembolic complications were observed in the long-term period in 5.4% of cases [30].

A study by Gunn et al. conducted at Turku University Hospital (Finland) involved 279 patients. Mean actuarial survival after implantation of the Björk–Shiley model in the aortic position was 19.8 years, the mean follow-up period was 19.2 years (maximum 34 years). Freedom from reoperation was 91.3% at 30 years. There were three cases of outlet strut fracture, two of which were fatal [31–34].

In 1977, a third-generation mechanical prosthesis was developed; St. Jude Medical Inc. (USA) produced a bicuspid carbon fiber prosthesis (Fig. 3) [35].

Fig. 3. St. Jude Medical mechanical heart valve model

The St. Jude Medical valve body and flaps were made of pyrolytic carbon, which has exceptional strength and low thrombogenicity. The flaps had an 85° opening angle to minimize flow turbulence. The low profile of this valve model and the rotation mechanism of the locking elements allowed for comfortable positioning of the prosthesis and minimized contact with subvalvular structures. With more than 84% of the valve area in the orifice, the average transprosthetic pressure gradient did not exceed 10 mmHg (in the aortic position), which was the lowest of all PHV models available at that time. However, the hinge units in this model were placed in the center of the prosthesis, which created three blood flows through the valve (Fig. 4) [14, 36, 37].

Based on an analysis of 25 years of experience with the St. Jude Medical valve, operative mortality was 4% and 9% when implanted in the aortic and mitral positions, respectively. Patient survival at 10 years after prosthesis

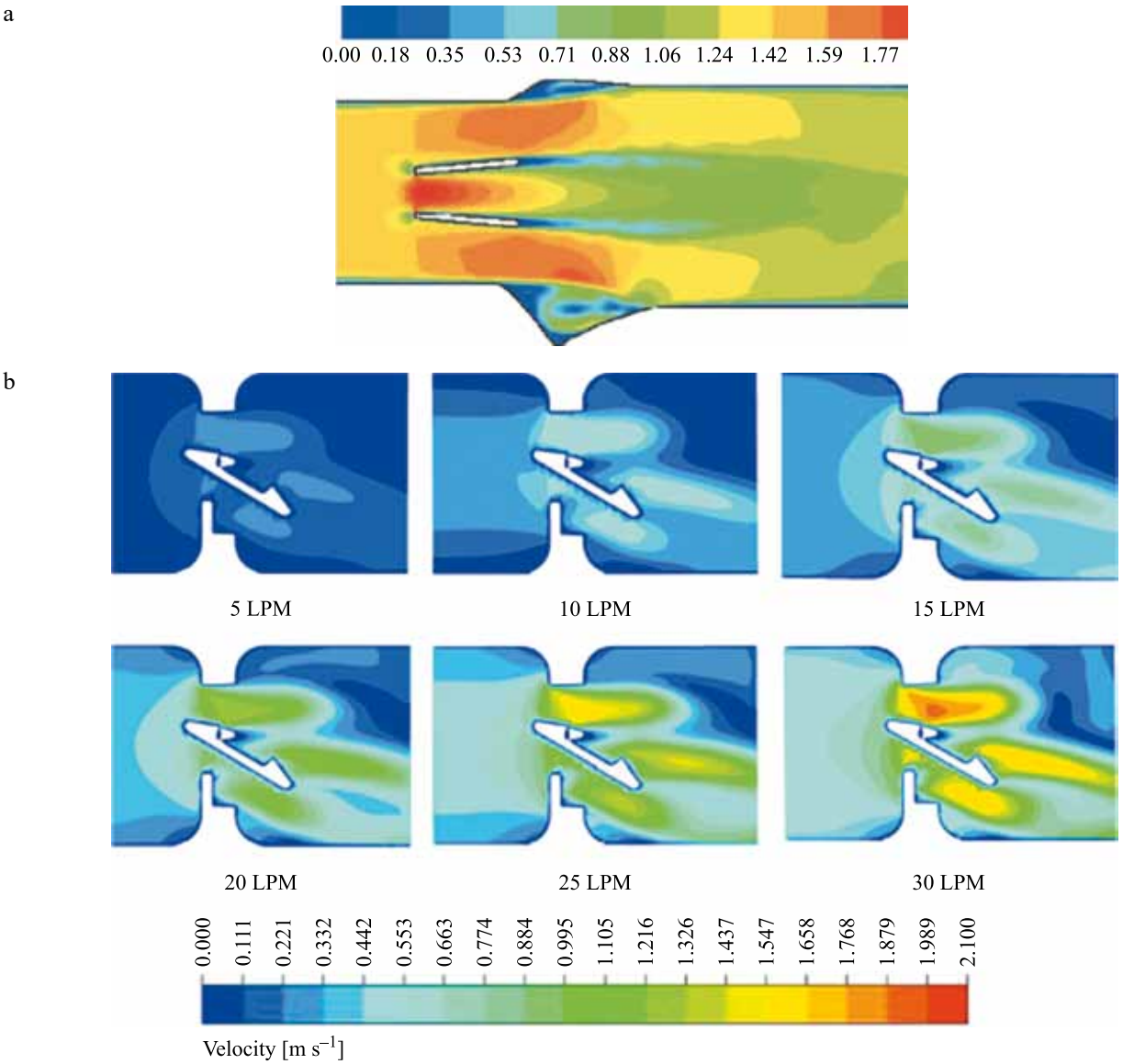

Fig. 4. Numerical modeling showing the distribution of flow velocity through a bicuspid valve model (a) and Björk–Shiley disc prosthesis (b) at a cardiac output of 5 L/min [14, 38]

was 57 ± 3% and 60 ± 2%, respectively [39]. According to Johnson et al. [40] the late actuarial survival of patients undergoing aortic valve replacement with St. Jude prosthesis was 62 ± 2%, 32 ± 2%, and 14 ± 3% after 10, 20, and 30 years, respectively. Thirty-year freedom from reoperation, thromboembolism, prosthetic valve thrombosis, bleeding, and endocarditis were 92 ± 2%, 79 ± 3%, 96 ± 1%, 56 ± 5%, and 92 ± 2%, respectively. A study by Rodrigues et al. [41] reported that valve-related mortality with the St. Jude prosthesis in the aortic valve replacement group was 11.3%, of which bleeding and thromboembolism accounted for 78%. In the mitral valve replacement group, 14% of deaths were valve-related, of which bleeding and thromboembolism accounted for 89%.

However, the St. Jude Medical valve model was also subjected to numerous modifications, resulting in the PHV On-X, a bicuspid mechanical heart valve prosthesis, the main feature of which was the presence of protrusions at the locking point of the flaps. In the open position, each leaf was deflected, forming an angle of 90° relative to the plane of the support ring. This property largely determined the laminar nature of flow through the valve.

The best results of On-X valve implantation among all known models of mechanical PHVs allowed us to explore the possibility of optimizing the safe international normalized ratio (INR) value in order to reduce the risk of complications associated with anticoagulant therapy [42]. The PROACT study investigated the safety of using different anticoagulant and antiplatelet therapy regimens after PHV On-X aortic valve replacement in low- and high-risk patients. Dual antiaggregant therapy in low-risk patients has been shown to result in a significantly higher incidence of neurological complications. This necessitated early termination of the study in this group [42, 43]. In the high-risk group (reduced left ventricular ejection fraction, increased left atrial volume, presence of atrial fibrillation), a reduced INR proved to be safe: no difference in survival and major cardiac events after 5 years [43].

Results of a study of survival after aortic valve replacement with different mechanical heart valve models are presented in Table.

The bicuspid aortic valve prosthesis design is used in prosthetic heart valves like ATS Medical Prosthesis, Sulzer CarboMedics, Sorin, MedInj, and others. Since the development of the bicuspid aortic valve, over 2.1 million implantations have been performed worldwide [36, 40]. However, the development of prostheses with the largest possible effective orifice to provide a hemodynamics that is close to that of the native valve remains a priority in PHV development. In our country, the improved PHV model is the domestic full-flow bicuspid valve MedEng-ST (Fig. 5) [5, 54].

The main advantage of the MedEng-ST valve is its design: the leaflets are fixed on hinge fasteners located on opposite sides of the ring, which helps to eliminate stagnant zones around the fasteners and reduces the likelihood of thromboembolic complications. A distinctive feature is the obturative element made in the form of two cylindrical segments, covering blood flow through the valve from the outside and providing blood flow centralization, minimal traumatization of formed elements, increasing the effective area of the valve orifice and reducing transprosthetic pressure gradient [5, 54].

Fig. 5. MedEng-ST mechanical heart valve model

Table

Actuarial survival after aortic valve replacement with different mechanical heart valve models

Literature	Valve model	Mean age, years, M ± SD	Survival (%)				
			1 year	5 years	10 years	15 years	25 years
Khan et al., 2001 [44]	St. Jude Medical	64.5 ± 12.9	91–95	71–87	39–73	17–61	N/A
Emery et al., 2005 [39]	St. Jude Medical	64 ± 13	N/A	N/A	N/A	N/A	<25
Toole et al., 2010 [45]	St. Jude Medical	56 ± 14	N/A	81	59	41	17
Tatsuishi W., 2015 [46]	St. Jude Medical	58.3 ± 11.7	N/A	96.2	92.7	88.8	N/A
Tossios et al., 2007 [47]	On-X	62.7	N/A	N/A	67.9	N/A	N/A
Carrier et al., 2006 [48]	CarboMedics	57 ± 12	N/A	83	70	62	N/A
Butchart et al., 2001 [49]	Medtronic-Hall	60 ± 11	N/A	N/A	64	45	N/A
Svennevig et al., 2007 [50]	Medtronic-Hall	54.3 ± 13.6	N/A	78.6	61.9	46.7	24.9
Ahn et al., 2007 [51]	Björk–Shiley Monostrut	34.5	96.8	N/A	91.1	86.5	N/A
Dietrich et al., 1989 [52]	Björk–Shiley Monostrut	60.5	N/A	98	N/A	N/A	N/A
Kallewaard et al., 2000 [36, 53]	Björk–Shiley convexo-concave	53.5 ± 13.9	92.1	83.7	68.7	55.0	N/A

Analysis of velocity distribution fields by finite element method using COMSOL Multiphysics program (Stockholm, Sweden) made it possible to clearly assess the degree of adaptation of PHV models and the ability to maintain laminar blood flow (Fig. 4 and 6). Unlike predecessor models (St. Jude Medical, Björk–Shiley), the MedEng-ST full-flow valve demonstrates excellent hemodynamic characteristics and flow laminarity of the profile.

Hemodynamic factors of thrombosis include the local hemodynamics features of the PHV, as well as individual parameters of patient hemodynamics [56]. Blood flow laminarity and the washability of all PHV components are among the main conditions for the effectiveness and safety of the PHV model. Reduced shear stress leads to stasis and increased blood coagulation [57], just as reduced cardiac output is a predictor of postoperative prosthetic valve thrombosis [58, 59]. Because of this, prosthetic valve thrombosis is almost 20 times more common in tricuspid valve replacement than mitral valve replacement. Similarly, PHV thrombosis in the mitral position is 2–3 times more common than prosthetic aortic valve thrombosis [60].

The flow characteristics of a prosthetic heart valve is considered the most important factor on which the safety and durability of a PHV model depend. However, an equally important condition determining the risk of thromboembolic complications is the surface properties of the materials from which the PHV components are made [61, 62]. Today, the main material used for manufacturing PHV locking elements is pyrocarbon. Widespread clinical use of pyrolytic carbon components for heart valve replacement began in October 1968, when Dr. Michael DeBakey implanted an aortic valve with a pyrolytic hollow-centered occlusion balloon with carbon ball [63]. After the first experience with the pyrolytic carbon component of PHVs, several million prosthetic mechanical valves made of this material have been implanted. The use of pyrolytic carbon in the fabrication of mechanical prosthetic heart valves was heralded as an "exceptional event" because the excellent durability, stability, and biocompatibility of pyrolytic carbon allowed the valves to be used for the lifetime of the patients [64]. Despite the modern pyrolytic carbon coating of prostheses, patients with implanted mechanical heart valves require lifelong anticoagulant therapy with vitamin K antagonists to prevent thromboembolic complications [65].

In contrast to healthy endothelium, which actively resists thrombosis, artificial surfaces promote clotting through a complex series of interrelated processes, including protein adsorption, platelet, leukocyte, and erythrocyte adhesion, thrombin generation, and complement activation. Rapid adsorption of plasma proteins onto artificial surfaces is thought to be the initiating event in thrombus formation because the protein layer modulates subsequent reactions of the coagulation cascade [66]. In turn, the dynamics of this process are related to the

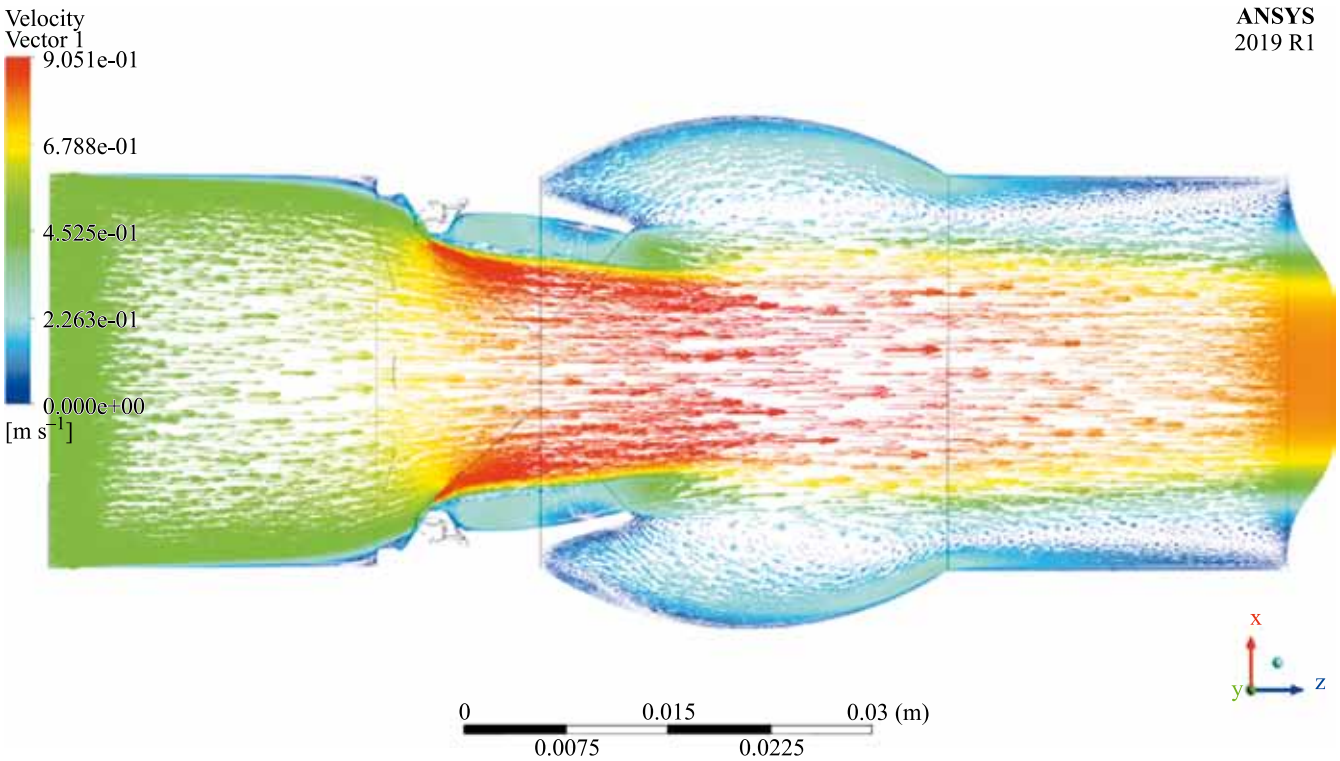

Fig. 6. Numerical modeling showing the distribution of flow velocity through the MedEng-ST model at a cardiac output of 5 L/min [55]

chemical and physical properties of the blood-contacting surface. Adsorbed proteins can form a monolayer surface with a 2–10 nm thickness, and their concentration on the surface can be 1000 times higher than in plasma [16, 67]. This process is particularly active on negatively charged surfaces [3] and appears to be flux independent [68]. At the same time, hydrophilicity is a key factor determining protein adsorption [67, 69]. The activation cascade is largely initiated by fibrinogen. Fibrinogen is one of the first plasma components to be adsorbed onto artificial surfaces. Other adhesive proteins, including Willebrand factor, also co-mediate platelet adhesion together with fibrinogen. Adsorbed fibrinogen is soon replaced by components of the contact system, including factor XII, high-molecular-weight kininogen, prekallikrein, and factor XI [70]. Activation of factor XII not only triggers thrombin generation through the intrinsic coagulation pathway, but also activates the complement system, which enhances thrombin generation [71–73]. Platelets adhered to the artificial surface of PHV are activated and they release thromboxane A2, ADP and other agonists of the hemostasis system. Leukocytes, especially neutrophils, also stimulate fibrinogen adsorption via CD11b/CD18 [74, 75]. In contrast to receptor-mediated adhesion of platelets and leukocytes to the protein monolayer, erythrocyte adhesion occurs passively [76]. Cross mechanisms between the complement and coagulation systems lead to formation of a platelet-fibrin network on the surface of prostheses [66].

Several studies have shown that platelet activation at the blood-material interface is dependent on a high albumin/fibrinogen (A/F) adsorption ratio (>1.00). That is, the higher the A/F ratio, the lower the number of adhered platelets [77]. Although pyrolytic carbon adsorbs albumin, concentration of fibrinogen on its surface is much higher and comparable to that in contact with silicone rubber [77]. However, in addition to protein absorption, the interaction energy and, consequently, the possibility of conformational changes in the protein layer are important in the development of subsequent thrombogenic reactions. Nyilas E. et. al, in the course of studying the interaction of blood plasma with foreign surfaces by measuring the heat of absorptions using the microcalorimetric method, found that this parameter for fibrinogen was significantly lower on pyrolytic carbon surfaces than on the known thrombogenic control (glass) surface up to completion of the formation of the first monolayer coating [78]. Furthermore, the measured net heats of adsorption of gamma globulin on pyrolytic carbon were about 15 times smaller than those on glass. As a result, the authors concluded that the low heat of adsorption on the foreign surface implies small interaction forces without conformational changes in the proteins that could activate the coagulation cascade. Thus, the protective protein layer formed after the first contact with blood ensures continuous exchange of protein molecules in an unchanged state, masking the pyrolytic carbon surface as a non-native one [79]. Approximately 3 months after implantation, the fibrin coating is replaced by a neointimal layer consisting of smooth muscle cells, elastin fibers, and endothelial cells. Over time, the neointimal layer matures and becomes more fibrous [80].

Chemical and physical surface properties of materials, such as hydrophobicity, hydrophilicity and surface energetics, determine biological reactions at the interface [81]. In addition, the topography of the biomaterial surface plays an essential role in determining bioinertness – it is this parameter that largely influences cell behavior (cell adhesion, proliferation, differentiation, and apoptosis). However, although this fact has long been known, the mechanisms underlying this process remain unexplored. It is known that cells can sense and respond to the nano-relief of a material using the so-called “contact guidance” [82, 83]. Superhydrophobic surfaces may provide an alternative approach to minimizing the thrombotic risk associated with blood-material interactions [84, 85]. These surfaces are fabricated by combining materials with low surface energy (typically <15 mN $\cdot$ m^{-1}) and texture [86]. It is known that these materials can reach contact angles as high as 120°. Microscopic air pockets existing in textured surfaces result in a composite liquid-air-solid interface and thus minimize the solid-liquid interface [86]. In addition to minimizing blood contact, material surface responses based on the Cassie-Baxter state can alter local hemodynamics through fluid slippage, potentially reducing the risk of hemolysis and platelet activation caused by increased shear stress [87].

The search for new synthetic materials that most closely mimic the properties of native endothelium has led to the emergence of a number of technologies for surface hypothrombogenic modification of implants. The machining and grinding operations of PHV parts do not exclude the appearance of cracks or surface defects, which can subsequently affect the service life of the product. Precise control of the surface modification process provided the possibility of applying a coating layer with the thickness necessary to eliminate surface defects caused by mechanical grinding, the main method of processing pyrolytic carbon [88, 89].

Surface modification of PHVs with coatings of various compositions can be used to improve selective properties (thromboresistance, anti-inflammatory effect) without changing their volumetric properties [90]. Over the past decade, diamond-like carbon (DLC) coating has been actively investigated for its possible use to improve the biocompatibility of synthetic materials, including PHVs [91–93]. In 1993, Dion et al. reported pronounced hypothrombogenic properties of DLC coatings during a study of the hemocompatibility of DLC-treated prosthetic heart valves made of titanium alloy T16A14B (SFERO-FII, St. Just Malmont, Frances) [94].

Since the early 2000s, DLC films have been shown to be bioinert, resistant to mechanical stress and corrosion, and non-cytotoxic to monocytes/macrophages, fibroblasts, and osteoblasts [95]. They have quite good hemocompatibility due to the optimal ratio of sp^3- and sp^2-hybridized carbon atoms [96]. In the last 5 years, due to some dissatisfaction with the results of biomedical testing of DLC coatings, publications on their physicochemical modification (in particular, with silicon and its oxides) improving the consumer properties of a-C:H:SiOx surface on medical materials and products have been accumulating [95]. Over the past few years, the study of cytotoxicity of a-C:H:SiOx coatings in relation to blood leukocytes, platelet adhesion, proinflammatory cytokine/chemokine production, as well as mechanical, anticorrosion and tribological properties has delivered impressive results. However, the safety and efficacy of such surface modification remains a subject of debate [91, 92, 97–99].

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170034, г. Тверь, пр. Чайковского, 9, оф. 514,
тел.: +7 (915) 730-10-37, +7 (910) 647-49-85
E-mail: triadatver@yandex.ru
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