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УЧРЕДИТЕЛИ: ОБЩЕРОССИЙСКАЯ ОБЩЕСТВЕННАЯ ОРГАНИЗАЦИЯ ТРАНСПЛАНТОЛОГОВ «РОССИЙСКОЕ ТРАНСПЛАНТОЛОГИЧЕСКОЕ ОБЩЕСТВО»

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DEPARTMENT OF TRANSPLANTOLOGY AND ARTIFICIAL ORGANS AT SECHENOV UNIVERSITY: 15 YEARS OF SUCCESS IN GRADUATE MEDICAL EDUCATION

Если развитие отечественной трансплантологии можно представить в виде дороги, по которой идем мы с вами, дорогие коллеги, то создание кафедры трансплантологии Сеченовского университета – это, без сомнения, заметная веха, мимо которой нельзя проследовать, не уделив внимания столь знаменательному событию. Тем более что в 2023 году исполняется 15 лет со дня ее основания. История кафедры тесно связана с развитием трансплантологии в Российской Федерации; для нашего профессионального поля это звено в единой цепи, часть пейзажа, без которого картина была бы неполной.

Развитие трансплантации и донорства органов в регионах России определяет необходимость в подготовке высококвалифицированных медицинских кадров, обладающих глубокими практическими и теоретическими знаниями в области трансплантологии и искусственных органов. В 2008 году мы обратились к руководству Сеченовского университета с предложением об открытии в составе лечебного факультета Первого государственного медицинского университета имени И.М. Сеченова первой в стране кафедры, где бы дисциплину «трансплантология и искусственные органы» преподавали в составе учебной программы «лечебное дело» студентам 6-го курса.

Ежегодно на кафедре по дисциплине «трансплантология и искусственные органы» прохо-

The development of Russian transplantology can be visualized as a road along which you and I, dear colleagues, are walking. The establishment of the Department of Transplantology at Sechenov University is, without doubt, a notable milestone that cannot be passed by without paying attention to such a significant event. More so because the 2023 marks 15 years since its foundation. The history of the department is closely connected with the development of transplantology in the Russian Federation. For our professional field, it is a link in a single chain, a part of the landscape, without which the picture would be incomplete.

The development of transplantation and organ donation programs in regions across Russia have created the need to train highly skilled medical personnel with profound practical and theoretical knowledge in the field of transplantology and artificial organs. In 2008, we approached the management of Sechenov University with a proposal to open Russia's first department, where the discipline "Transplantology and Artificial Organs" would be taught to year 6 students as part of the "Medicine" curriculum under the Faculty of Medicine.

At this department, over 1000 students are trained annually under the "Transplantology and Artificial Organs" discipline. The discipline includes the following modules: Fundamentals of transplantology and artificial organs; Problems of organ donation; Clinical heart transplantation; Clinical liver transplantation; Clinical pancreas transplantation;

дят обучение более 1000 студентов. Дисциплина включает в себя следующие модули: основы трансплантологии и искусственных органов; проблемы органного донорства; клиническая трансплантация сердца; клиническая трансплантация печени; клиническая трансплантация поджелудочной железы; клиническая трансплантация почки; трансплантационная иммунология и иммуносупрессия; инновационные решения и перспективы в области трансплантологии и искусственных органов.

Профессорско-преподавательский состав кафедры представлен высококвалифицированными сотрудниками: членом-корреспондентом РАН, 8 докторами и 5 кандидатами наук. Эффективное сочетание практической работы и образовательной деятельности на базе современного научно-клинического центра позволяет интегрировать новые возможности в сферу образования, науки и медицины. В 2018 году вышел в свет учебник «Трансплантология и искусственные органы», который составлен с учетом требований Федерального государственного образовательного стандарта и учебного плана и базируется на многолетнем опыте преподавания этой дисциплины в ведущем медицинском вузе России.

Плодотворное сотрудничество кафедры трансплантологии и искусственных органов Сеченовского университета с ведущим профильным научно-клиническим учреждением – ФГБУ «НМИЦ трансплантологии и искусственных органов имени академика В.И. Шумакова» Минздрава России – позволило создать образовательную платформу, способствующую подготовке высококвалифицированных кадров, развитию высокотехнологичной медицины, обеспечению доступной и качественной медицинской помощью граждан России.

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

Clinical kidney transplantation; Transplantation immunology and immunosuppression; Innovative solutions and perspectives in the field of transplantology and artificial organs.

The teaching staff at the department comprises 15 high-caliber specialists: 1 associate fellow of the Russian Academy of Sciences, 8 professors of medicine and 5 holders of doctorate degrees in medicine. Effective combination of practical work and educational activities at this modern research and clinical center allows integrating new opportunities in the field of education, science and medicine. In 2018, the textbook "Transplantology and Artificial Organs" was published. The book was compiled keeping in mind the requirements of the Federal State Educational Standard and the curriculum. It is based on many years of experience in teaching this discipline at Russia's leading medical university.

Fruitful cooperation between the Department of Transplantology and Artificial Organs at Sechenov University and the Shumakov National Medical Research Center of Transplantology and Artificial Organs, a leading specialized research and clinical institution, has facilitated the creation of an educational platform that promotes the training of highly skilled talent for the development of high-tech medicine, and the provision of affordable and top-quality medical care to Russian citizens.

Sincerely,

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

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ORGAN DONATION AND TRANSPLANTATION IN THE RUSSIAN FEDERATION IN 2022

15th Report from the Registry of the Russian Transplant Society

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Objective: to monitor the current trends and developments in organ donation and transplantation in the Russian Federation based on data from the year 2022. **Materials and methods.** Heads of organ transplant centers were surveyed through questionnaires. Data control was done using the information accounting system of the Russian Ministry of Health. We performed a comparative analysis of data obtained over years from various federal subjects of the Russian Federation and transplant centers. **Results.** Based on data retrieved from the National Registry in 2022, 46 kidney, 31 liver and 16 heart transplant programs were existing in the Russian Federation as of the year 2022. Organ donation activity in 2022 was 5.2 per million population (p.m.p.), with a 73.7% multi-organ procurement rate and an average of 2.8 organs procured from one effective donor. In 2022, 2,555 organ transplants were performed in the Russian Federation, which included 1,562 kidney, 659 liver and 310 heart transplants. Same year, the number of transplant surgeries performed in the Russian Federation increased by 10.0% compared to 2021. In Moscow, organ donation activity was 26.3 p.m.p. The city of Moscow and the Moscow Oblast alone had a total of 12 transplant centers, accounting for 52.6% of all kidney transplants and 64.8% of all extrarenal transplants in the country. The number of organ recipients in the Russian Federation exceeds 150 p.m.p. **Conclusion.** The geographic distribution of transplant centers in the Russian Federation continues to expand. Five new centers were opened in 2022. Over the past year, the number of effective donors and organ transplants increased in the country. The resource potential of medical institutions has not been exhausted and this is set to further increase the number of organ transplants performed. Moscow is the powerhouse of Russian transplantology. However, other regional leaders have since appeared in the Russian Federation, such as in Kemerovo, Kazan, Rostov-on-Don, Tyumen, Irkutsk, and Volzhsky. In the Russian Federation, priority is being given to pediatric transplant care. It is expedient to implement a complex of measures aimed at identifying potential recipients.

Keywords: organ donation, kidney transplantation, liver transplantation, heart transplantation, lung transplantation, transplant center, waiting list, registry, Shumakov National Medical Research Center of Transplantology and Artificial Organs.

INTRODUCTION

Current trends and developments in organ donation and transplantation in Russia are monitored via the National Registry under the auspices of a specialized transplantology commission jointly created by the Russian Ministry of Health and the Russian Transplant Society. Previous reports have been published in 2009–2022 [1–13].

Information contained in the Registry is provided to the following international registries:

– International Registry of Organ Donation and Transplantation (IRODaT);
– Registry of the European Renal Association – European Dialysis and Transplant Association (ERA–EDTA Registry);
– Registries of the International Society for Heart and Lung Transplantation (ISHLT Registries).

Since 2016, the National Registry has served as a tool for ensuring quality control and data collection integrity in the information system used for registering donated human organs and tissues, donors and recipients. The system operates under executive order No. 355n of the Russian Ministry of Health, dated June 8, 2016.

Annual reports of the register are not only statistical data for the reporting period, but also their systematic analysis with an assessment of the current state of transplantation care in the Russian Federation, trends and prospects for further development of this branch of healthcare.

Since 2019, the register is used to monitor the implementation of the departmental target program "Organ

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Donation and Transplantation in the Russian Federation", approved by the order of the Ministry of Health of Russia from June 4, 2019 No. 365 (from 2022 – a set of process measures).

Data for the Registry is collected via questionnaires administered to appropriate officials at all transplant centers in the Russian Federation. There is a comparative analysis of all data gathered over years from Russian regions, transplant centers and from international registries.

The working group would like to thank all regular and new participants in the Registry who have provided data, as well as the Russian Ministry of Health, and the Central Research Institute for Healthcare Organization and Informatization.

TRANSPLANT CENTERS

There are transplant centers in 37 federal subjects of the Russian Federation (see Fig. 1).

In 2022, kidney transplantation (KiT) was performed in 46 centers, liver transplantation (LiT) in 31, heart transplantation (HT) in 16, pancreas transplantation (PnT) in 3, lung transplantation (LnT) in 4.

In 2022, various transplant interventions were performed in 57 medical institutions. Of these, 18 were federal institutions, including 11 institutions of the Russian Ministry of Health, 2 institutions of the Russian Ministry of Science and Higher Education, 4 institutions of the Federal Biomedical Agency, 1 institution of the Russian Ministry of Defense, and 39 are institutions run by federal subjects of the Russian Federation.

In the new territories of the Russian Federation, in 2022 there was one transplant center in the Donetsk People's Republic at the Donetsk Clinical Territorial Medical Association of the DPR Ministry of Health of the DNR (Donetsk). Over the past year, there were 4 living-donor kidney transplants.

In 2022, 2,555 organ transplants were performed in Russia – 258 were pediatric transplants (see Tables 1 and 2). The number of organ transplants in the Russian Federation increased by 10.2% (+237) compared to 2021.

In 2022, 159 (in February) to 264 (in April) organ transplants were performed monthly, about 210 on average (see Fig. 2).

In the past year, 92 to 163 KiT, 42 to 72 LiT and 21 to 32 HT per month were performed in the Russian Federation.

Table 1

Organ donation and transplantation in the Russian Federation in 2022

Indicator	Number (units)
Organ donation	
Total number of organ donors	1,149
Deceased donors	763
Living (related) donors	386
Organ transplantation	
Total number of organs transplanted	2,555
share of pediatric transplants	258
Kidney	1,562
from deceased donors	1,334
from living-related donors	228
share of pediatric transplants	118
Liver	659
from deceased donor	501
from living-related donors	158
share of pediatric transplants	129
Heart	308
share of pediatric transplants	10
Heart-lung	2
share of pediatric transplants	1
Lungs	14
Pancreas	10

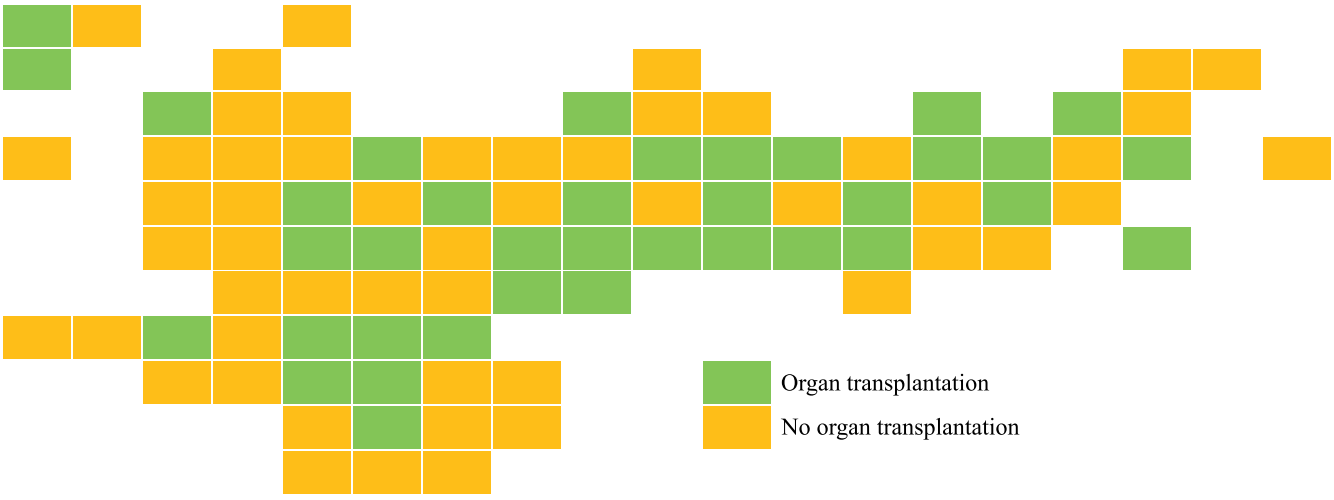

Fig. 1. Geographic distribution of organ transplant centers in Russia in 2022

Based on data obtained from the Federal Registry for High-Tech Medical Care, 2,186 (85.5%) organ transplant surgeries were performed in 2022, using funds from the compulsory medical insurance system that were allocated for provision of high-tech medical care for organ transplantation; there were 2,052 (88.5%) of such surgeries in 2021; see Fig. 3. Another 369 (14.5%) organ transplants were performed using funds from the federal subjects of the Russian Federation and from the federal budget.

Since 2010, when funding was included in the Registry as an indicator, the number of organ transplants performed using the funds allocated for provision of high-tech medical care for organ transplant has increased 2.7-fold. Meanwhile, the proportion of organ transplants performed using these funds has increased by 27.5%.

The financial costs per unit of high-tech medical care for transplantation in 2022 were approved by the Government of the Russian Federation on December 28, 2021 via Resolution No. 2505.

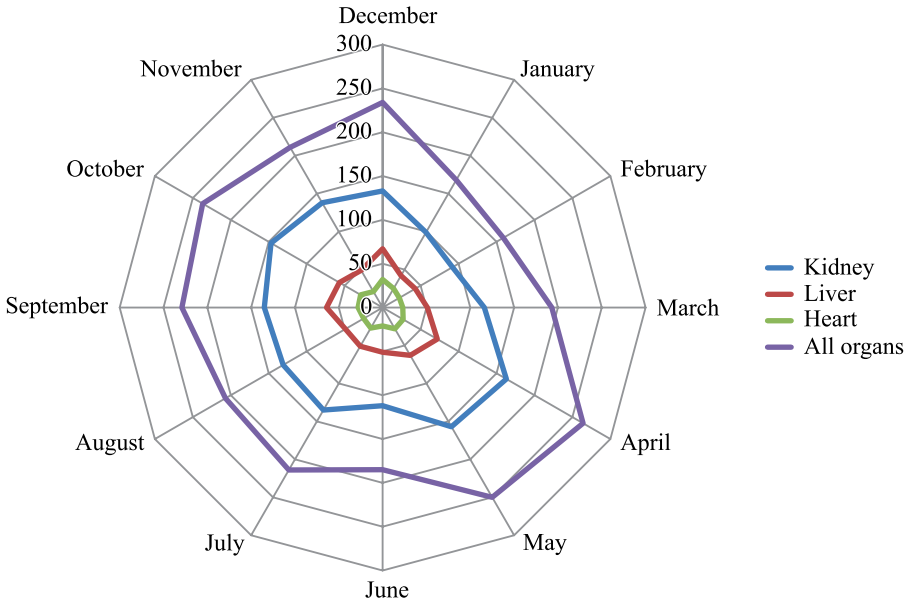

Fig. 2. Organ transplantation by month in 2022

Table 2

Transplant activity in the Russian Federation in 2022

№	Transplant center, region, federal district	Total	Kidney (total)	Kidney (cadaver)	Kidney (living related)	Liver (total)	Liver (cadaver)	Liver (living related)	Heart	Pancreas	Lungs	Heart-lungs	Small intestine
1	2	3	4	5	6	7	8	9	10	11	12	13	14
1.1	Shumakov National Medical Research Center of Transplantology and Artificial Organs, **Moscow, Central Federal District**	**654**	**266**	171	95	**159**	63	96	**212**	**5**	**10**	**2**	**0**
1.2	Volzhsky Branch of Shumakov National Medical Research Center of Transplantology and Artificial Organs, **Volzhsky, Southern Federal District**	**45**	**36**	20	16	**7**	7	0	**2**	**0**	**0**	**0**	**0**
2	Lopatkin Research Institute of Urology and Interventional Radiology, a branch of the National Medical Research Center for Radiology, **Moscow, Central Federal District**	**50**	**50**	43	7	**0**	0	0	**0**	**0**	**0**	**0**	**0**
3	Russian Children's Clinical Hospital, **Moscow, Central Federal District**	**32**	**32**	25	7	**0**	0	0	**0**	**0**	**0**	**0**	**0**
4	Petrovsky National Research Centre of Surgery, **Moscow, Central Federal District**	**38**	**25**	6	19	**13**	0	13	**0**	**0**	**0**	**0**	**0**

Continuation table 2

1	2	3	4	5	6	7	8	9	10	11	12	13	14
5	Burnazyan Federal Medical and Biophysical Center, **Moscow, Central Federal District**	**48**	**13**	11	2	**35**	11	24	**0**	**0**	**0**	**0**	**0**
6	Bakulev Scientific Center of Cardiovascular Surgery, **Moscow, Central Federal District**	**2**	**0**	0	0	**0**	0	0	**2**	**0**	**0**	**0**	**0**
7	National Medical Research Center for Children's Health, **Moscow, Central Federal District**	**18**	**18**	8	10	**0**	0	0	**0**	**0**	**0**	**0**	**0**
8	Botkin Hospital, **Moscow, Central Federal District**	**149**	**108**	107	1	**41**	41	0	**0**	**0**	**0**	**0**	**0**
9	Sklifosovsky Research Institute of Emergency Care, **Moscow, Central Federal District**	**369**	**251**	250	1	**107**	105	2	**4**	**3**	**4**	**0**	**0**
10	Moscow Clinical Scientific Center, **Moscow, Central Federal District**	**24**	**0**	0	0	**24**	24	0	**0**	**0**	**0**	**0**	**0**
11	Vladimirsky Moscow Regional Research and Clinical Institute, **Moscow Oblast, Central Federal District**	**62**	**39**	38	1	**23**	23	0	**0**	**0**	**0**	**0**	**0**
12	Federal Clinical Center for High Medical Technologies, Federal Biomedical Agency (119), **Moscow Oblast, Central Federal District**	**20**	**20**	16	4	**0**	0	0	**0**	**0**	**0**	**0**	**0**
13	St. Joasaphus Belgorod Regional Clinical Hospital, **Belgorod, Central Federal District**	**13**	**9**	9	0	**4**	4	0	**0**	**0**	**0**	**0**	**0**
14	Voronezh Regional Clinical Hospital No. 1, **Voronezh, Central Federal District**	**7**	**7**	6	1	**0**	0	0	**0**	**0**	**0**	**0**	**0**
15	Tula Regional Clinical Hospital, **Tula, Central Federal District**	**4**	**4**	3	1	**0**	0	0	**0**	**0**	**0**	**0**	**0**
16	Ryazan Regional Clinical Hospital, **Ryazan, Central Federal District**	**12**	**11**	10	1	**1**	1	0	**0**	**0**	**0**	**0**	**0**
17	Stavropol Regional Clinical Hospital, **Stavropol, North Caucasian Federal District**	**3**	**0**	0	0	**3**	3	0	**0**	**0**	**0**	**0**	**0**
18	Ochapovsky Regional Clinical Hospital No. 1, **Krasnodar, Southern Federal District**	**48**	**31**	29	2	**9**	9	0	**8**	**0**	**0**	**0**	**0**
19	Volzhsky Regional Center of Urology, **Volzhsky, Southern Federal District**	**7**	**7**	3	4	**0**	0	0	**0**	**0**	**0**	**0**	**0**
20	Rostov Regional Clinical Hospital, **Rostov-on-Don, Southern Federal District**	**56**	**34**	34	0	**16**	16	0	**5**	**1**	**0**	**0**	**0**
21	Russian Research Center of Radiology and Surgical Technologies, **St. Petersburg, Northwestern Federal District**	**16**	**0**	0	0	**16**	16	0	**0**	**0**	**0**	**0**	**0**
22	Almazov National Medical Research Centre, **St. Petersburg, Northwestern Federal District**	**28**	**0**	0	0	**0**	0	0	**28**	**0**	**0**	**0**	**0**
23	Pavlov University, **St. Petersburg, Northwestern Federal District**	**45**	**35**	32	3	**10**	10	0	**0**	**0**	**0**	**0**	**0**
24	St. Petersburg Research Institute of Emergency Medicine, **St. Petersburg, Northwestern Federal District**	**24**	**24**	24	0	**0**	0	0	**0**	**0**	**0**	**0**	**0**
25	Mariinskaya Hospital, **St. Petersburg, Northwestern Federal District**	**16**	**16**	14	2	**0**	0	0	**0**	**0**	**0**	**0**	**0**
26	St. Luke's Clinical Hospital, **St. Petersburg, Northwestern Federal District**	**7**	**7**	5	2	**0**	0	0	**0**	**0**	**0**	**0**	**0**
27	Kirov Military Medical Academy, **St. Petersburg, Northwestern Federal District**	**18**	**0**	0	0	**18**	17	1	**0**	**0**	**0**	**0**	**0**
28	Leningrad Regional Clinical Hospital, **St. Petersburg, Northwestern Federal District**	**33**	**33**	33	0	**0**	0	0	**0**	**0**	**0**	**0**	**0**
29	Volosevich First City Clinical Hospital, **Arkhangelsk, Northwestern Federal District**	**3**	**3**	2	1	**0**	0	0	**0**	**0**	**0**	**0**	**0**

End of table 2

1	2	3	4	5	6	7	8	9	10	11	12	13	14
30	Meshalkin National Medical Research Center, **Novosibirsk, Siberian Federal District**	**10**	**0**	0	0	**1**	1	0	**9**	**0**	**0**	**0**	**0**
31	State Novosibirsk Regional Clinical Hospital, **Novosibirsk, Siberian Federal District**	**78**	**35**	27	8	**43**	24	19	**0**	**0**	**0**	**0**	**0**
32	Research Institute for Complex Issues of Cardiovascular Diseases, **Kemerovo, Siberian Federal District**	**11**	**0**	0	0	**0**	0	0	**11**	**0**	**0**	**0**	**0**
33	Belyaev Kemerovo Regional Clinical Hospital, **Kemerovo, Siberian Federal District**	**75**	**75**	72	3	**0**	0	0	**0**	**0**	**0**	**0**	**0**
34	Regional Clinical Hospital for Emergency Medical Care, **Kemerovo, Siberian Federal District**	**7**	**0**	0	0	**7**	7	0	**0**	**0**	**0**	**0**	**0**
35	Irkutsk Regional Clinical Hospital, **Irkutsk, Siberian Federal District**	**35**	**18**	18	0	**16**	16	0	**1**	**0**	**0**	**0**	**0**
36	Altai Regional Clinical Hospital, **Barnaul, Siberian Federal District**	**21**	**19**	19	0	**2**	2	0	**0**	**0**	**0**	**0**	**0**
37	Federal Center for Cardiovascular Surgery, **Krasnoyarsk, Siberian Federal District**	**20**	**18**	15	3	**2**	2	0	**0**	**0**	**0**	**0**	**0**
38	Krasnoyarsk Regional Clinical Hospital, **Krasnoyarsk, Siberian Federal District**	**31**	**18**	18	0	**9**	9	0	**4**	**0**	**0**	**0**	**0**
39	Sverdlovsk Regional Clinical Hospital No. 1, **Yekaterinburg, Ural Federal District**	**30**	**20**	19	1	**7**	6	1	**3**	**0**	**0**	**0**	**0**
40	Chelyabinsk Regional Clinical Hospital, **Chelyabinsk, Ural Federal District**	**22**	**15**	14	1	**4**	4	0	**3**	**0**	**0**	**0**	**0**
41	Regional Clinical Hospital No. 1, **Tyumen, Ural Federal District**	**33**	**30**	29	1	**1**	1	0	**2**	**0**	**0**	**0**	**0**
42	District Clinical Hospital, **Khanty-Mansiysk, Ural Federal District**	**8**	**5**	4	1	**2**	2	0	**1**	**0**	**0**	**0**	**0**
43	Samara State Medical University, **Samara, Volga Federal District**	**43**	**41**	39	2	**2**	2	0	**0**	**0**	**0**	**0**	**0**
44	Saratov State Medical University, **Saratov, Volga Federal District**	**7**	**7**	0	7	**0**	0	0	**0**	**0**	**0**	**0**	**0**
45	Regional Clinical Hospital, **Saratov, Volga Federal District**	**4**	**4**	4	0	**0**	0	0	**0**	**0**	**0**	**0**	**0**
46	Volga Regional Medical Center, **Nizhny Novgorod, Volga Federal District**	**24**	**14**	11	3	**9**	7	2	**0**	**1**	**0**	**0**	**0**
47	Republican Clinical Hospital, **Kazan, Volga Federal District**	**138**	**83**	80	3	**55**	55	0	**0**	**0**	**0**	**0**	**0**
48	Interregional Clinical Diagnostic Center, **Kazan, Volga Federal District**	**8**	**0**	0	0	**0**	0	0	**8**	**0**	**0**	**0**	**0**
49	Republican Clinical Hospital, **Ufa, Volga Federal District**	**49**	**39**	39	0	**10**	10	0	**0**	**0**	**0**	**0**	**0**
50	Republican Cardiology Clinic, **Ufa, Volga Federal District**	**5**	**0**	0	0	**0**	0	0	**5**	**0**	**0**	**0**	**0**
51	Perm Regional Clinical Hospital, **Perm, Volga Federal District**	**3**	**3**	2	1	**0**	0	0	**0**	**0**	**0**	**0**	**0**
52	Municipal Clinical Hospital for Emergency Medical Care No. 1, **Orenburg, Volga Federal District**	**16**	**16**	11	5	**0**	0	0	**0**	**0**	**0**	**0**	**0**
53	Republican Hospital No. 1 – National Center of Medicine, **Yakutsk, Far Eastern Federal District**	**2**	**2**	2	0	**0**	0	0	**0**	**0**	**0**	**0**	**0**
54	Semashko Republican Clinical Hospital, **Ulan-Ude, Far Eastern Federal District**	**3**	**3**	0	3	**0**	0	0	**0**	**0**	**0**	**0**	**0**
55	Primorsky Regional Clinical Hospital No. 1, **Vladivostok, Far Eastern Federal District**	**15**	**12**	12	0	**3**	3	0	**0**	**0**	**0**	**0**	**0**
56	Regional Clinical Hospital No. 1, **Khabarovsk, Far Eastern Federal District**	**2**	**2**	0	2	**0**	0	0	**0**	**0**	**0**	**0**	**0**
Total		**2551**	**1558**	**1334**	**224**	**659**	**501**	**158**	**308**	**10**	**14**	**2**	**0**

ORGAN DONATION

In 2022, donor programs were implemented in 34 federal subjects of the Russian Federation.

Over the past year, new donor programs were launched in 2 federal subjects of the Russian Federation:

– in Khabarovsk Krai, from a living donor;
– in Perm Krai, from a deceased donor.

The rate of increase in donor activity in the Russian Federation in 2021 was higher by 17.3% than planned by the departmental target program "Organ Donation and Transplantation in the Russian Federation", approved by executive order No. 365 of the Russian Ministry of Health dated June 4, 2019.

In 2022, the proportion of effective deceased organ donors >60 years of age was 16.0% (see Fig. 4). Male donors were 63.3%, females were 36.7%.

Donor activity per population of the regions implementing donor programs (100.0 million) amounted to 7.6 p.m.p. (see Tables 4 and 5).

Moscow posted the highest donor activity – 26.3 p.m.p. (23.7 in 2021). In Kemerovo Oblast, donor activity exceeded 15.0 effective donors p.m.p. (15.8). In two more federal subjects of the Russian Federation, Republic of Tatarstan and the Tyumen Oblast, donor activity exceeded 10.0 p.m.p.

In 2022, an increase in donor activity was observed in 20 federal subjects of the Russian Federation; Kemerovo Oblast, the Republic of Tatarstan, Tyumen Oblast, Leningrad Oblast, St. Petersburg, Irkutsk Oblast, Altai Krai, Primorsky Krai, and Chelyabinsk Oblast showed the most dynamic growth (by ≥40.0%).

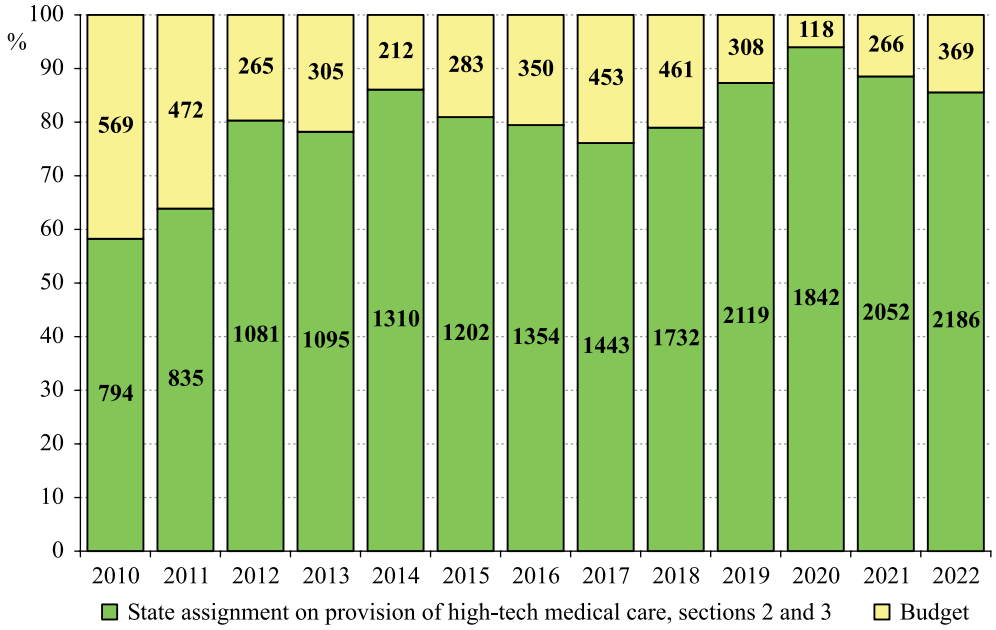

Fig. 3. Funding for organ transplantation in the Russian Federation in 2010–2022

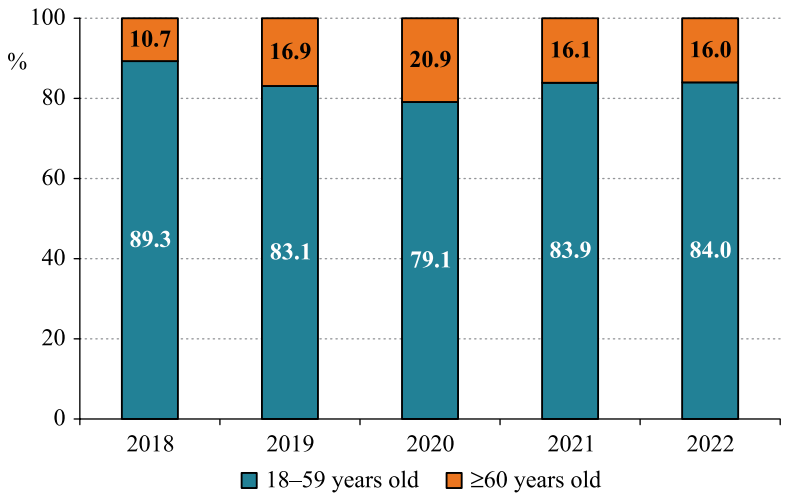

Fig. 4. Age structure of effective organ donors in 2018–2022

Eleven federal subjects of the Russian Federation witnessed a drop in donor activity; donor activity in Ryazan Oblast, Sverdlovsk Oblast and Stavropol Krai was worse than in other regions (≥30% decrease).

Moscow and Moscow Oblast alone accounted for 47.4% (362) of effective donors in 2022.

There were 725 effective brain-dead donors, accounting for 95.0% of the total pool of effective donors (see Fig. 5). In 26 federal subjects of the Russian Federation, the centers worked only with brain-dead donors.

In Kemerovo Oblast, the share of effective donors diagnosed with brain death increased, but still remains lower than in other federal subjects (70.7% in 2022 and 64.3% in 2021).

There were 562 multi-organ procurements in 2022, accounting for 73.6% of the total number of procurements. In 17 federal subjects, the share of multi-organ procurements was ≥70.0%.

The donor resource is underutilized in Ryazan Oblast, Voronezh Oblast, Kemerovo Oblast, Tyumen Oblast, Samara Oblast, Saratov Oblast, Primorsky Krai – the share of multi-organ donors is <50.0%).

Moscow and Moscow Oblast alone accounted for 294 multi-organ donors (38.5% of the total number of multi-organ donors) in the country in 2022.

The average number of organs procured from one donor in 2022 was 2.8 (3.0 in 2021). Donor kidney utilization rate was 87.4% (90.7% in 2021).

In 2022, the number of organs (kidney, part of the liver) procured from living related donors reached 386 – 33.6% of the total number of procurements (1,149).

Table 3

Indicators associated with organ donation activity in the regions of the Russian Federation in 2022

S/N	Region	Organ Donation Coordinating Center	Population (million)	Number of active donor bases	Effective donors (absolute, per million population)		including brain-dead donors (absolute, %)		including multi-organ donors (absolute, %)	
1	2	3	4	5	6	7	8	9	10	11
1	Moscow	Botkin Hospital	12.6	21	332	26.3	313	94.3	268	80.7
2	Moscow Oblast	Vladimirsky Moscow Regional Research Clinical Institute	7.8	13	30	3.8	30	100.0	26	86.7
3	Belgorod Oblast	St. Joasaphus Belgorod Regional Clinical Hospital	1.5	1	5	3.3	5	100.0	4	80.0
4	Voronezh Oblast	Voronezh Regional Clinical Hospital No. 1	2.3	3	3	1.3	3	100.0	0	0.0
5	Tula Oblast	Tula Regional Clinical Hospital	1.4	1	3	2.1	3	100.0	3	100.0
6	Ryazan Oblast	Ryazan Regional Clinical Hospital	1.1	1	7	6.4	6	85.7	3	42.9
7	Krasnodar Krai	Ochapovsky Regional Clinical Hospital No. 1	5.7	1	17	3.0	16	94.1	15	88.2
8	Volgograd Oblast	Volzhsky Branch of Shumakov National Medical Research Center of Transplantology and Artificial Organs	2.5	3	8	3.2	8	100.0	7	87.5
9	Rostov Oblast	Rostov Regional Clinical Hospital	4.2	1	21	5.0	21	100.0	19	90.5
10	Stavropol Krai	Stavropol Regional Clinical Hospital	2.8	1	3	1.1	3	100.0	2	66.7
11	St. Petersburg	St. Petersburg Research Institute of Emergency Medicine	5.4	6	43	8.0	43	100.0	36	83.7
12	Leningrad Oblast	Leningrad Regional Clinical Hospital	1.9	1	17	8.9	17	100.0	14	82.4
13	Arkhangelsk Oblast	Volosevich First City Clinical Hospital	1.1	1	3	2.7	3	100.0	3	100.0
14	Novosibirsk Oblast	State Novosibirsk Regional Clinical Hospital	2.8	4	19	6.8	18	94.7	18	94.7
15	Kemerovo Oblast	Belyaev Kemerovo Regional Clinical Hospital	2.6	10	41	15.8	29	70.7	19	46.3
16	Irkutsk Oblast	Irkutsk Regional Clinical Hospital	2.4	2	15	6.3	15	100.0	9	60.0
17	Altai Krai	Altai Regional Clinical Hospital	2.3	1	10	4.3	10	100.0	5	50.0
18	Krasnoyarsk Krai	Krasnoyarsk Regional Clinical Hospital	2.9	3	10	3.4	10	100.0	10	100.0
19	Sverdlovsk Oblast	Sverdlovsk Regional Clinical Hospital No. 1	4.3	2	10	2.3	10	100.0	6	60.0
20	Chelyabinsk Oblast	Chelyabinsk Regional Clinical Hospital	3.4	1	9	2.6	9	100.0	7	77.8

End of table 3

1	2	3	4	5	6	7	8	9	10	11
21	Tyumen Oblast	Regional Clinical Hospital No. 1	1.5	3	16	10.7	16	100.0	5	31.3
22	Khanty-Mansi Autonomous Okrug – Yugra	District Clinical Hospital	1.7	3	3	1.8	3	100.0	2	66.7
23	Samara Oblast	Samara State Medical University	3.1	4	23	7.4	22	95.7	4	17.4
24	Saratov Oblast	Regional Clinical Hospital	2.4	1	7	2.9	7	100.0	2	28.6
25	Nizhny Novgorod Oblast	Volga Regional Medical Center	3.2	4	7	2.2	7	100.0	6	85.7
26	Republic of Tatarstan	Republican Clinical Hospital	3.9	3	52	13.3	52	100.0	44	84.6
27	Republic of Bashkortostan	Kuvatov Republican Clinical Hospital	4.0	6	20	5.0	20	100.0	12	60.0
28	Orenburg Oblast	Municipal Clinical Hospital for Emergency Medical Care No. 1	1.9	2	5	2.6	5	100.0	5	100.0
29	Primorsky Krai	Primorsky Regional Clinical Hospital No. 1	1.9	1	7	3.7	7	100.0	2	28.6
30	Perm Krai	Perm Regional Clinical Hospital	2.5	1	1	0.4	1	100.0	0	0.0
31	The Republic of Sakha (Yakutia)	Republican Hospital No. 1, National Center of Medicine	1.0	1	1	1.0	1	100.0	0	0.0
32	Departmental program of the Federal Biomedical Agency of the Russian Federation	Burnazyan Federal Medical and Biophysical Center	–	2	2	–	2	100.0	2	100.0
33	Departmental program of the Federal Biomedical Agency of the Russian Federation	Federal Siberian Research and Clinical Center	–	3	13	–	10	76.9	4	30.8
		Total	**145.5**	**111**	**763**	**5.2**	**725**	**95.0**	**562**	**73.7**

Table 4

Rating of regions by donor activity in 2022

S/N	Federal Subject of the Russian Federation (Region)	Population in 2022 (million)	Number of effective donors (per million population)	
			2022	2021
1	Moscow	12.6	26.3	23.7
2	Kemerovo Oblast	2.6	15.8	10.8
3	Republic of Tatarstan	3.9	13.3	9.0
4	Tyumen Oblast	1.5	10.7	5.3
5	Leningrad Oblast	1.8	8.9	6.3
6	St. Petersburg	5.4	8.0	4.6
7	Samara Oblast	3.2	7.4	7.7
8	Novosibirsk Oblast	2.8	6.8	5.4
9	Ryazan Oblast	1.1	6.4	10.0
10	Irkutsk Oblast	2.4	6.3	3.3
11	Republic of Bashkortostan	4.1	5.0	5.3
12	Rostov Oblast	4.2	5.0	5.0
13	Altai Krai	2.3	4.3	3.0
14	Moscow Oblast	7.7	3.8	4.7
15	Primorsky Krai	1.9	3.7	1.6
16	Krasnoyarsk Krai*	2.9	3.4	4.1
17	Belgorod Oblast	1.5	3.3	1.3
18	Volgograd Oblast	2.5	3.2	4.0

S/N	Federal Subject of the Russian Federation (Region)	Population in 2022 (million)	Number of effective donors (per million population)	
			2022	2021
19	Krasnodar Krai	5.6	3.0	2.3
20	Saratov Oblast	2.4	2.9	2.5
21	Arhangelsk Oblast	1.1	2.7	0.9
22	Orenburg Oblast	1.9	2.6	2.1
23	Chelyabinsk Oblast	3.5	2.6	0.9
24	Sverdlovsk Oblast	4.3	2.3	3.3
25	Nizhny Novgorod Oblast	3.2	2.2	2.2
26	Tula Oblast	1.4	2.1	2.9
27	Khanty-Mansi Autonomous Okrug – Yugra	1.7	1.8	1.2
28	Voronezh Oblast	2.3	1.3	1.3
29	Stavropol Krai	2.8	1.1	1.8
30	The Republic of Sakha (Yakutia)	1	1.0	0.0
31	Perm Krai	2.5	1.0	0.4
	Russia (85 federal subjects of the Russian Federation)	145.5	5.2	4.5

Note: The donor program of the Federal Siberian Research and Clinical Center, Krasnoyarsk is excluded.

Table 5

Deceased organ donors (effective donors) in 2006–2022

S/N	Region	2006	2007		2008		2009		2010		2011		2012		2013		2014		2015		2016		2017		2018		2019		2020		2021		2022	
		Number of effective donors	Number of effective donors	Year-over-year change (abs.)	Number of effective donors	Year-over-year change (abs.)	Number of effective donors	Year-over-year change (abs.)	Number of effective donors	Year-over-year change (abs.)	Number of effective donors	Year-over-year change (abs.)	Number of effective donors	Year-over-year change (abs.)	Number of effective donors	Year-over-year change (abs.)	Number of effective donors	Year-over-year change (abs.)	Number of effective donors	Year-over-year change (abs.)	Number of effective donors	Year-over-year change (abs.)	Number of effective donors	Year-over-year change (abs.)	Number of effective donors	Year-over-year change (abs.)	Number of effective donors	Year-over-year change (abs.)	Number of effective donors	Year-over-year change (abs.)	Number of effective donors	Year-over-year change (abs.)	Number of effective donors	Year-over-year change (abs.)
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
1	Moscow	87	126	+39	135	+9	136	+1	151	+15	135	–16	111	–24	125	+14	151	+26	142	–9	183	+41	195	+12	218	+23	277	+59	263	–14	298	+35	332	+34
2	Moscow Oblast	24	45	+21	59	+14	52	–7	71	+19	82	+11	61	–21	56	–5	51	–5	44	–7	39	–5	75	+36	68	–7	41	–27	21	–20	36	+15	30	–6
3	Belgorod Oblast		2	+2	3	+1	2	–1	5	+3	6	+1	3	–3	1	–2	2	+1	5	+3	4	–1	4	0	4	0	4	0	2	–2	2	0	5	+3
4	Voronezh Oblast	6	2	–4	8	+6	2	–6	0	–2	1	+1	6	+5	6	0	5	–1	7	+2	4	–3	1	–3	8	+7	8	0	4	–4	3	–1	3	0
5	Tula Oblast																										2	+2	3	+1	4	+1	3	–1
6	Ryazan Oblast																								2	+2	13	+11	6	–7	11	+5	7	–4
7	Ivanovo Oblast																														1	+1	0	–1
8	Krasnodar Krai						3	+3	39	+36	52	+13	42	–10	41	–1	23	–18	25	+2	24	–1	19	–5	20	+1	23	+3	13	–10	13	0	17	+4
9	Volgograd Oblast	5	0	–5	11	+11	15	+4	16	+1	17	+1	19	+2	15	–2	18	+3	8	–10	8	0	9	+1	9	0	10	+1	10	0	10	0	8	–2
10	Rostov Oblast																		1	+1	7	+6	13	+6	19	+6	21	+2	18	–3	21	+3	21	0
11	Stavropol Krai																								2	+2	3	+1	13	+10	5	–8	3	–2
12	St. Petersburg	30	45	+15	47	+2	47	0	41	–6	34	–7	22	–12	13	–9	23	+10	31	+8	29	–2	31	+2	34	+3	53	+19	25	–28	25	0	43	+18
13	Leningrad Oblast	12	8	–4	11	+3	11	0	13	+2	10	–3	10	0	10	0	9	–1	7	–2	12	+5	11	–1	15	+4	7	–8	11	+4	12	+1	17	+5
14	Arkhangelsk Oblast																								5	+5	5	0	1	–4	1	0	3	+2
15	Novosibirsk Oblast	17	11	–6	18	+7	29	+11	35	+6	25	–10	20	–4	17	–3	11	–6	14	+3	9	–5	14	+5	17	+3	23	+6	15	–8	15	0	19	+4
16	Kemerovo Oblast	16	13	–3	18	+5	18	0	22	+4	12	–10	26	+14	26	0	31	+5	28	–3	34	+6	22	–12	30	+8	40	+10	27	–13	28	+1	41	+13
17	Irkutsk Oblast				4	+4	6	+2	10	+4	9	–1	8	–1	6	–2	9	+3	4	–5	3	–1	2	–1	7	+5	16	+9	16	0	8	–8	15	+7
18	Omsk Oblast	10	15	+5	13	–2	19	+6	19	0	14	–5	11	–3	14	+3	16	+2	11	–5	4	–7	4	0	3	–1	2	–1	2	0	0	–2	0	0
19	Altai Krai														3	+3	5	+2	4	–1	4	0	8	+4	8	0	8	0	9	+1	7	–2	10	+3
20	Krasnoyarsk Krai																3	+3	6	+3	18	+12	27	+9	16	Note	13	–3	10	–3	12	+2	10	–2
21	Sverdlovsk Oblast	14	13	–1	12	–1	13	+1	14	+1	15	+1	14	–1	18	+4	23	+5	18	–5	15	–3	22	+7	24	+2	24	0	6	–18	14	+8	10	–4

End of table 5

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
22	Chelyabinsk Oblast								6	+6	2	–4	7	+5	6	–1	10	+4	9	–1	11	+2	8	–3	4	–4	4	0	3	–1	3	0	9	+6
23	Tyumen Oblast																						4	+4	13	+9	13	0	5	–8	8	+3	16	+8
24	Khanty-Mansi Autonomous Okrug – Yugra																						3	+3	4	+1	5	+1	3	–2	2	–1	3	+1
25	Samara Oblast	4	17	+13	24	+7	18	–6	20	+2	21	+1	19	–2	21	+2	20	–1	18	–2	26	+8	28	+2	23	–5	25	+2	24	–1	24	0	23	–1
26	Saratov Oblast														4	+4	7	+3	7	0	7	0	7	0	8	+1	10	+2	0	–10	6	+6	7	+1
27	Nizhny Novgorod Oblast						7	+7	11	+4	12	+1	10	–2	8	–2	12	+4	10	–2	11	+1	10	–1	12	+2	12	0	5	–7	7	+2	7	0
28	Republic of Tatarstan		3	+3	1	–2	3	+2	12	+9	16	+4	9	+7	6	–3	6	0	4	–2	1	–3	3	+2	4	+1	15	+11	21	+6	35	+14	52	+17
29	Republic of Bashkortostan								2	+2	7	+5	14	+7	18	+4	19	+1	14	+5	20	+6	22	+2	20	–2	24	+4	18	–6	21	+3	20	–1
30	Orenburg Oblast																		3	+3	8	+5	9	+1	8	–1	11	+3	1	–10	4	+3	5	+1
31	The Republic of Sakha (Yakutia)																				2	+2	4	+2	4	0	3	–1	0	–3	0	0	1	+1
32	Primorsky Krai																														3	+3	7	+4
33	Perm Krai																																1	+1
34	Burnazyan Federal Medical and Biophysical Center, Moscow														6	+6	11	+5	14	+3	16	+2	9	–7	5	–4	1	–4	1	0	3	+2	2	–1
35	Burnazyan Federal Medical and Biophysical Center, Krasnoyarsk																								24	Note	16	–8	8	–8	10	+2	13	+3
	TOTAL in the Russian Federation	**225**	**300**	**+75**	**364**	**+64**	**381**	**+17**	**487**	**+106**	**470**	**–17**	**412**	**–58**	**420**	**+8**	**465**	**+45**	**434**	**–31**	**499**	**+53**	**565**	**+78**	**639**	**+74**	**732**	**+93**	**564**	**–168**	**652**	**+88**	**763**	**+111**

Note: The donor activity of the Federal Siberian Research and Clinical Center, Krasnoyarsk is presented as a separate program.

In 2021, the number of organ procurements from living related donors was 364–35.8% of the total number of procurements (1,016).

KIDNEY TRANSPLANTATION

In 2022, a total of 1,562 KiT were performed (see Fig. 6).

Compared to the year 2021, the number of KiT increased by 12.9% (+178).

A new KiT program was launched in Khabarovsk Krai (Regional Clinical Hospital No. 1, Khabarovsk).

In 2022, there were 1,334 deceased-donor KiT and 228 living-related-donor KiT (see Fig. 6).

Table 6 and Fig. 7 show KiT centers that performed the highest number of KiT in 2022.

The rating primarily demonstrates the leadership and sustainability of the transplant programs at leading transplant centers in Moscow, which in turn is a result of the effective work by the Moscow Coordinating Center for Organ Donation.

The positive dynamics of transplant programs in the Republic of Tatarstan and Kemerovo Oblast, the sustainability and volume of KiT programs in Samara Oblast, Republic of Bashkortostan and Moscow Oblast, and further development of pediatric KiT program at Shumakov National Research Center (Moscow) and

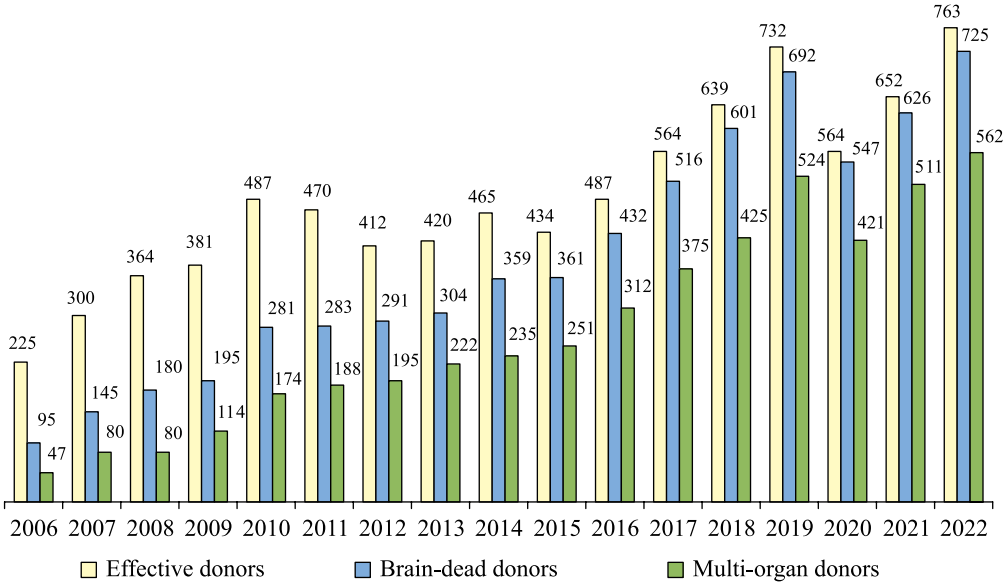

Fig. 5. Structure of effective organ donors in the Russian Federation in 2006–2022

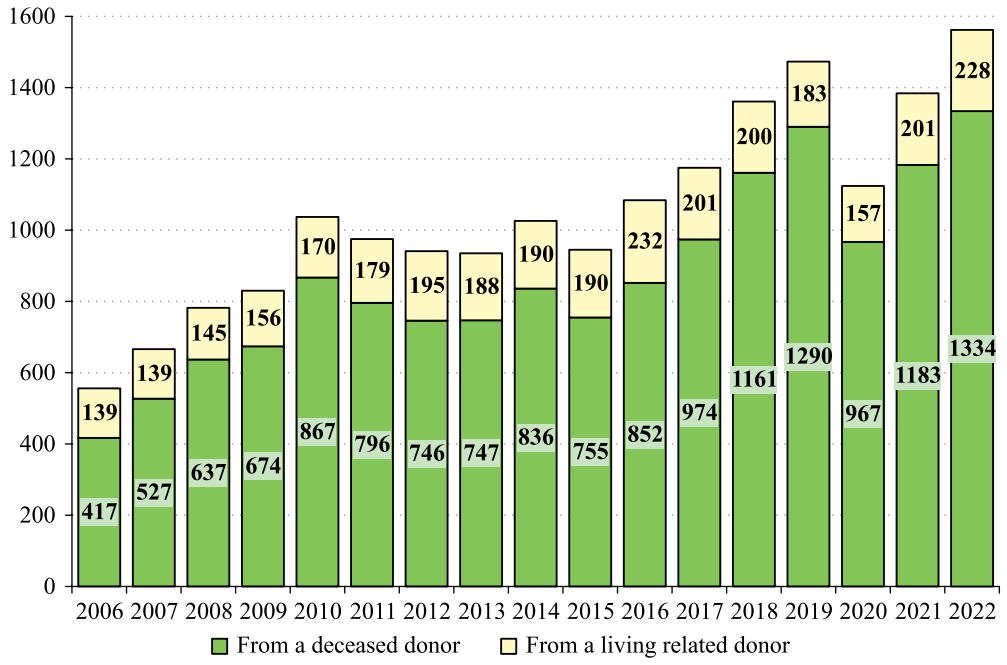

Fig. 6. Kidney transplantation in the Russian Federation in 2006–2022

Russian Children's Clinical Hospital (Moscow) should be noted. Shumakov National Medical Research Center of Transplantology and Artificial Organs, Moscow (Shumakov Center) plays a leading role in the living-related KiT program, performing 111 transplants (49.5% of the total number of related KiT in Russia).

In 2022, 6 KiT centers performed more than 50 surgeries during the year: Shumakov Center (302), Sklifosovsky Research Institute of Emergency Care (251), Botkin Hospital (108), Republican Clinical Hospital, Kazan (83), Belyaev Kemerovo Regional Clinical Hospital (75), and Research Institute of Urology (50). Lopatkin Research Institute of Urology (50). Ten transplant centers performed from 30 to 49 operations during the year; another 12 centers performed from 15 to 29.

In 2022, 35 transplant centers (76.1%) performed related-donor KiT, with a total of 228 transplants performed. The average utilization of living kidney donation

Table 6

Leaders by number of kidney transplants performed

Rank	Leaders in terms of number of kidney transplants performed	Number of kidney transplants in 2022
1	Shumakov National Medical Research Center of Transplantology and Artificial Organs, Moscow	266
2	Sklifosovsky Research Institute of Emergency Care, Moscow	251
3	Botkin Hospital, Moscow	108
4	Republican Clinical Hospital, Kazan	83
5	Belyaev Kemerovo Regional Clinical Hospital, Kemerovo	75
6	Lopatkin Research Institute of Urology and Interventional Radiology, a branch of the National Medical Research Center for Radiology, Moscow	50
7	Samara State Medical University, Samara	41
8	Kuvatov Republican Clinical Hospital, Ufa	39
9	Vladimirsky Moscow Regional Research and Clinical Institute, Moscow Oblast	39
10	Volzhsky Branch of Shumakov National Medical Research Center of Transplantology and Artificial Organs, Volzhsky	36
	TOTAL	**988**
	63.4% of the total number of kidney transplants performed in the Russian Federation (1,558)	

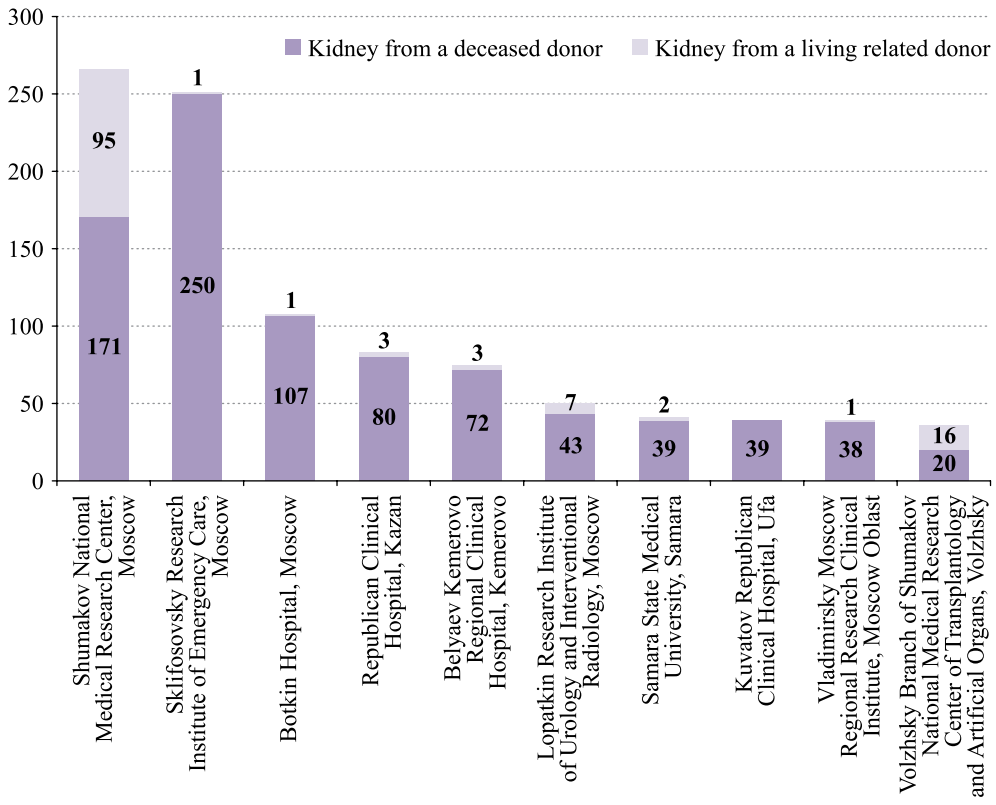

Fig. 7. Leaders by number of kidney transplants performed

in 2022 was 14.6% of the total number of KiT (14.5% in 2021).

Pediatric KiT (minors ≤17 years of age) in 2022 were performed at 8 centers, and a total of 118 transplants were performed. Among the institutions performing it were Shumakov Center (56), Russian Children's Clinical Hospital (32), and National Medical Research Center for Children's Health (18); see Fig. 8.

EXTRARENAL ORGAN TRANSPLANTATION

In 2022, there were 308 HT, of which 10 were pediatric transplants and 2 heart-lung transplants (at Shumakov Center).

Heart transplants were performed in 16 centers. New HT programs were launched in 2 federal subjects of the Russian Federation:

– Volgograd Oblast (at the branch of Shumakov Center in Volzhsky),
– Irkutsk Oblast (at Irkutsk Regional Clinical Hospital in Irkutsk).

Shumakov Center (Moscow) accounts for 69.7% (216, including 2 heart-lung transplants) of the total number of HT in the Russian Federation. The HT program in this center continues to drive the level of availability of this type of transplant care in the country.

Apart from the Shumakov Center, more than 10 heart transplants in Russia were performed at Almazov National Medical Research Centre (28) and at the Research Institute for Complex Issues of Cardiovascular Diseases (11). Another 5 transplant centers performed from 5 to 9 HT heart transplants: Ochapovsky Regional Clinical Hospital No. 1 (Krasnodar), Rostov Regional Clinical Hospital (Rostov-on-Don), Meshalkin National Medical Research Center (Novosibirsk), Interregional Clinical Diagnostic Center (Kazan), and Republican Clinical Hospital (Ufa). The remaining 8 (50.0%) performed less than 5 HT in the year.

LnT in 2021 were performed at 2 transplant centers. A total of 13 LnT and 2 heart-lung transplants were performed: 10 lung and 2 heart-lung transplants at Shumakov Center, 4 LnT at Sklifosovsky Research Institute of Emergency Care.

Table 7 and Fig. 9 show the thoracic organ transplant centers that performed the highest number of heart-lung transplants in 2022.

In 2022, a total of 659 liver transplants were performed, including 129 pediatric transplants. LiT were performed in 31 centers.

Two new liver transplantation programs were launched in 2022 – at Samara State Medical University, Samara and at Primorsky Regional Clinical Hospital No. 1, Vladivostok.

In 2022, 2 transplant centers performed more than 100 liver transplants: Shumakov Center (166) and Sklifosovsky Research Institute of Emergency Care (107). Six other transplant centers performed 20 or more LiT each: Republican Clinical Hospital, Kazan (55), State Novosibirsk Regional Clinical Hospital, Novosibirsk (43), Botkin Hospital (41), Burnazyan Federal Medical and Biophysical Center (35), Moscow Clinical Scientific Center (24), and Vladimirsky Moscow Regional Research Clinical Institute (23).

Table 8 and Fig. 10 show the liver transplant centers where the largest number of LiT were performed in 2022.

The rating primarily demonstrates the leadership and sustainability of the transplant programs at leading transplant centers in Moscow, which in turn is a result of the effective work by the Moscow Coordinating Center for Organ Donation and the use of the technology of transplantation of a part of the liver from a living related donor. The positive dynamics of transplant programs in the Republic of Tatarstan and Novosibirsk Oblast, and the leading role of pediatric living related LiT at Shumakov Center (Moscow) should all be noted.

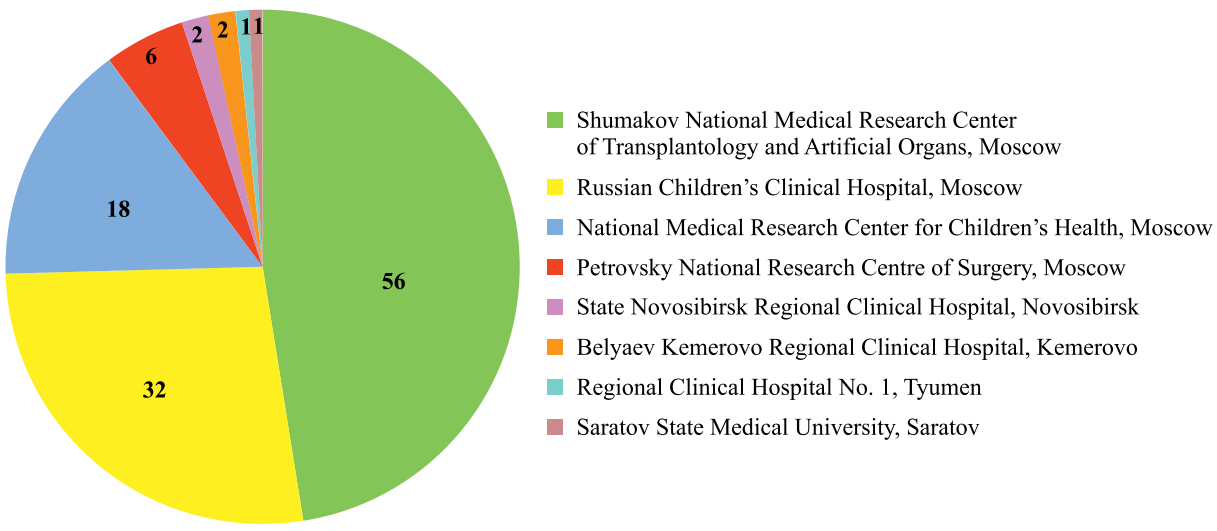

Fig. 8. Pediatric kidney transplantation in the Russian Federation in 2022

Related LiT were performed at 8 centers; living-related transplants accounted for 24.0% (158 transplant surgeries).

In 2022, 129 pediatric LiT were (mostly in tender-age children). Pediatric LiT were performed in 3 centers: in Shumakov Center (111), Petrovsky National Research Centre of Surgery (13), and State Novosibirsk Regional Clinical Hospital in Novosibirsk (5).

Pancreas transplants in 2022 were performed at 4 transplant centers: Shumakov Center (5), Sklifosovsky Research Institute of Emergency Care (3), Rostov Regional Clinical Hospital, Rostov-on-Don (1), and Volga Regional Medical Center, Nizhny Novgorod (1). A total of 10 pancreas transplant surgeries were performed (10 in 2021), all of them being kidney-pancreas transplants.

Thus, there were 993 extrarenal transplants performed in 2022 or 38.9% of the total number of 2,555 (934, 40.3% in 2021). Transplant centers in Moscow and Moscow Oblast alone accounted for 64.8% (644) of extrarenal organ transplants in 2022.

Table 7

Medical institutions that performed ≥5 heart transplants

Rank	Centers that performed ≥5 heart transplants	Number of heart transplants in 2022
1	Shumakov National Medical Research Center of Transplantology and Artificial Organs, Moscow	216*
2	Almazov National Medical Research Centre, St. Petersburg	28
3	Research Institute for Complex Issues of Cardiovascular Diseases, Kemerovo	11
4	Meshalkin National Medical Research Center, Novosibirsk	9
5	Ochapovsky Regional Clinical Hospital No. 1, Krasnodar	8
6	Interregional Clinical Diagnostic Center, Kazan	8
7	Rostov Regional Clinical Hospital, Rostov-on-Don	5
8	Republican Cardiology Clinic, Ufa	5
	TOTAL	**290**
	93.5% of the total number of heart transplants performed in the Russian Federation (310)	

* including two heart-lung transplants.

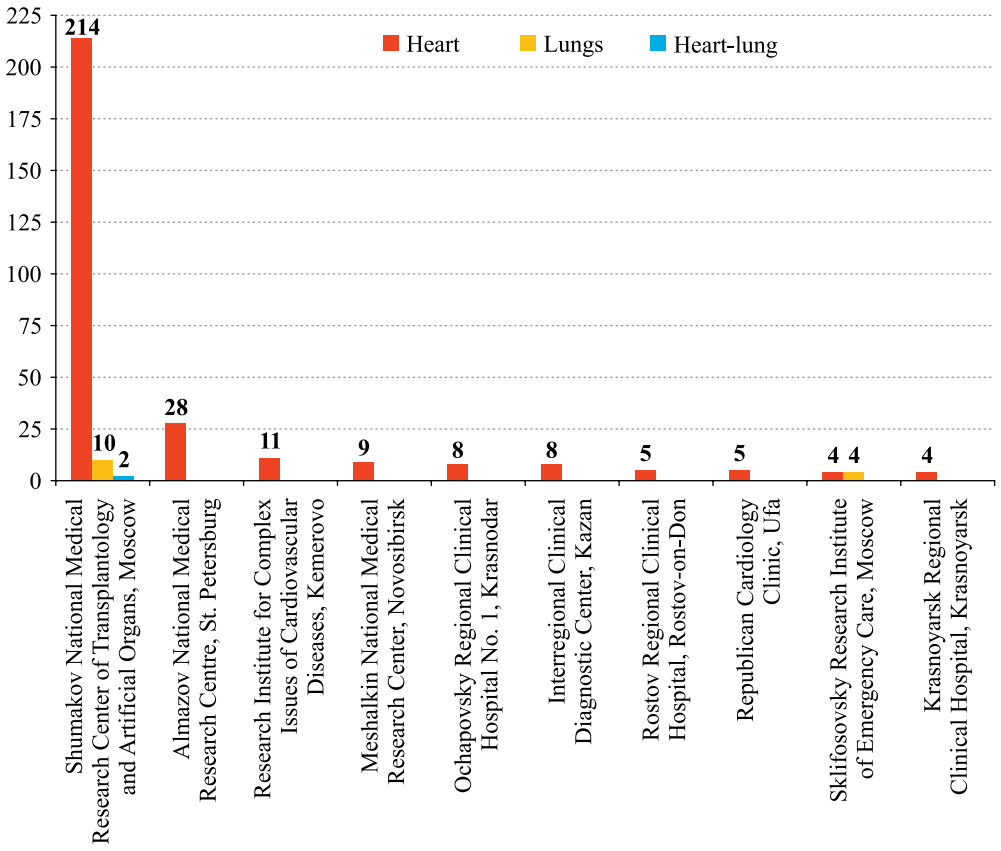

Fig. 9. Medical institutions that performed ≥5 heart transplants

During the follow-up period from 2006, the number of extrarenal organ transplants in the Russian Federation increased by 887 (9.4-fold); see Figs. 11 and 12.

Table 9 presents the number of organ transplants performed in the Russia Federation from 2006 to 2022.

ORGAN TRANSPLANT RECIPIENTS

As of December 2022, there were 21,969 organ transplant recipients in the Russian Federation, excluding the new Russian territories (151.0 p.m.p.); see Table 10.

Over the 9 years of observation, the number of organ recipients in the Russian Federation increased 2.6-fold (by 13,416 patients); the number of kidney recipients is estimated at 13,721 (94.3 p.m.p.); liver recipients, 4,294 (29.5 p.m.p.); heart recipients, 1,916 (13.2 p.m.p.).

CONCLUSION

In 2022, the main objectives and trends in the development of organ donation and transplantation in the federal subjects of the Russian Federation remained the same and did not lose their relevance:

– expanding the geographic footprint and number of transplant centers;

Table 8

Leaders in terms of number of liver transplants performed

Rank	Leaders in terms of number of liver transplants performed	Number of liver transplants in 2022
1	Shumakov National Medical Research Center of Transplantology and Artificial Organs, Moscow	159
2	Sklifosovsky Research Institute of Emergency Care, Moscow	107
3	Republican Clinical Hospital, Kazan	55
4	State Novosibirsk Regional Clinical Hospital, Novosibirsk	43
5	Botkin Hospital, Moscow	41
6	Burnazyan Federal Medical and Biophysical Center, Moscow	35
7	Moscow Clinical Scientific Center, Moscow	24
8	Vladimirsky Moscow Regional Research and Clinical Institute, Moscow Oblast	23
	TOTAL	**487**
	73.9% of the total number of liver transplants performed in the Russian Federation (659)	

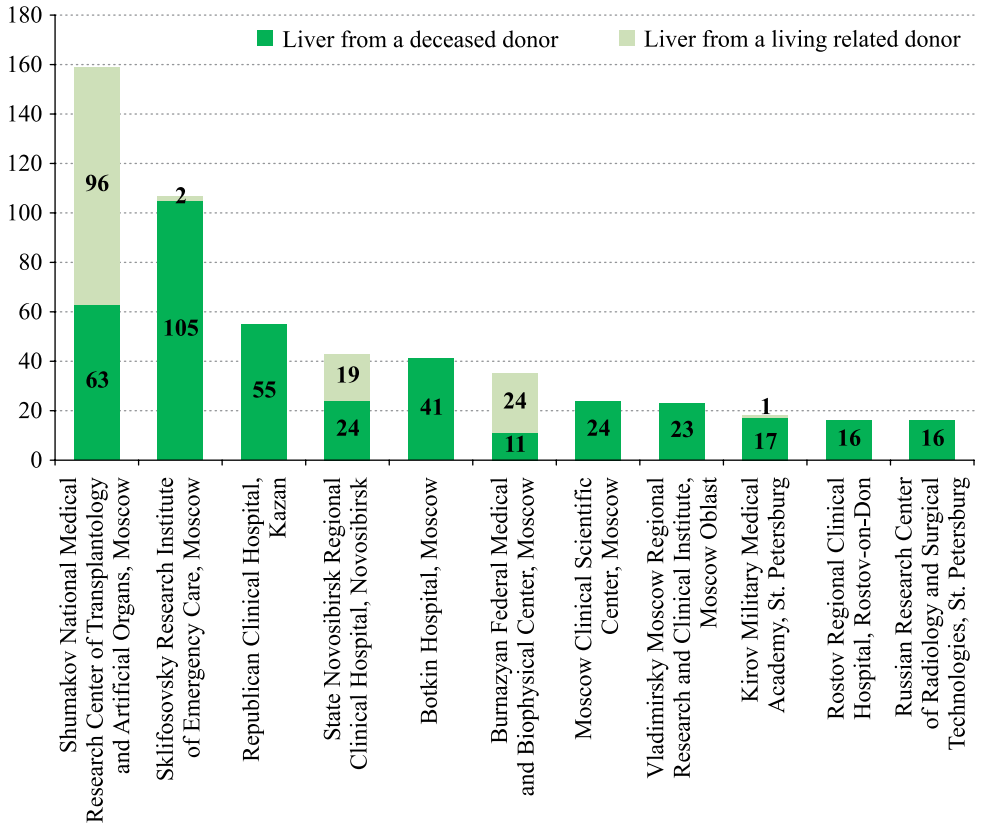

Fig. 10. Leaders in terms of number of liver transplants performed

– effectively identifying patients in need and their inclusion in the organ transplant waiting list;
– increasing the number of deceased organ donors in accordance with the available donor resource, increasing the proportion of brain-dead donors and multi-organ donors;
– increasing the number of organ transplants in accordance with the real need of the population;
– prioritizing the provision of transplantation care to the pediatric population;
– 100.0% coverage of medical monitoring, including drug supply, for transplant recipients.

Five new organ donation and transplantation programs were opened in 2022:

Two living-related-donor kidney transplants were performed in Khabarovsk Krai (Regional Clinical Hospital No. 1, Khabarovsk).

Two heart transplants were performed in Volgograd Oblast (a branch of Shumakov Center in Volzhsky),

A heart transplant was performed in Irkutsk Oblast (Irkutsk Regional Clinical Hospital, Irkutsk).

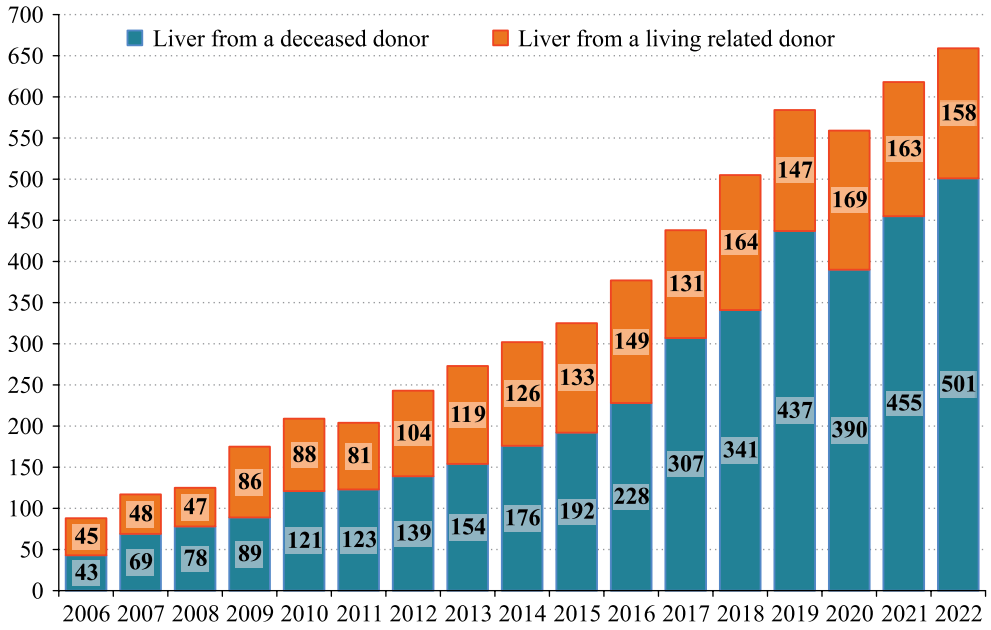

Fig. 11. Heart transplantation in the Russian Federation in 2006–2022

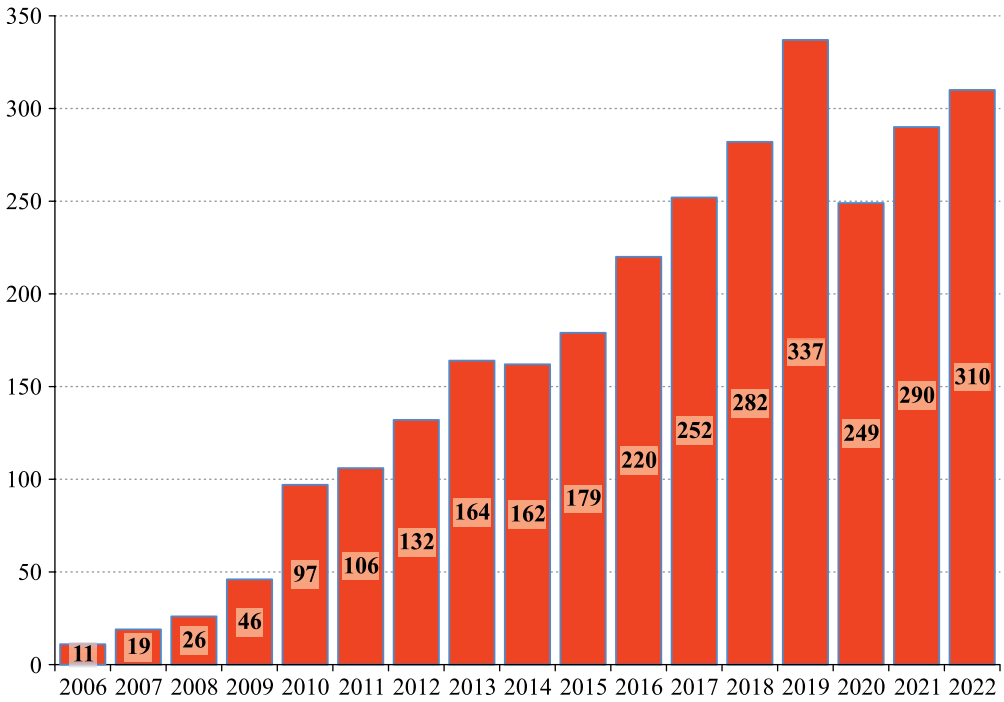

Fig. 12. Liver transplantation in the Russian Federation in 2006–2022

Table 9

Organ transplantation in the Russian Federation in 2006–2022

S/N	Organ	2006	2007		2008		2009		2010		2011		2012		2013		2014		2015		2016		2017		2018		2019		2020		2021		2022	
		Absolute number	Absolute number	Year-over-year change	Absolute number	Year-over-year change	Absolute number	Year-over-year change	Absolute number	Year-over-year change	Absolute number	Year-over-year change	Absolute number	Year-over-year change	Absolute number	Year-over-year change	Absolute number	Year-over-year change	Absolute number	Year-over-year change	Absolute number	Year-over-year change	Absolute number	Year-over-year change	Absolute number	Year-over-year change	Absolute number	Year-over-year change	Absolute number	Year-over-year change	Absolute number	Year-over-year change	Absolute number	Year-over-year change
1	Kidney (total)	556	666	+110	782	+116	830	+48	1037	+207	975	–62	941	–34	935	–6	1026	+91	945	–81	1084	+139	1175	+91	1361	+186	1473	+112	1124	–349	1384	+258	1562	+180
2	*from a deceased donor*	417	527	+110	637	+110	666	+29	867	+201	796	–71	746	–50	747	+1	836	+89	755	–81	852	+97	974	+122	1161	+187	1290	+129	967	–323	1183	+216	1334	+151
3	*from a living related donor*	139	139	0	145	+6	156	+11	170	+14	179	+9	195	+16	188	–7	190	+2	190	0	232	+42	201	–31	200	–1	183	–17	157	–26	201	+44	228	+27
4	Liver (total)	88	117	+29	125	+8	175	+50	209	+34	204	–5	243	+39	272	+29	302	+30	325	+23	378	+53	438	+60	505	+67	584	+79	559	–25	618	+59	659	+41
5	*from a deceased donor*	43	69	+26	78	+9	89	+11	121	+32	123	+2	139	+16	154	+15	176	+22	192	+16	229	+37	307	+78	341	+34	437	+96	390	–47	455	+65	501	+46
6	*from a living related donor*	45	48	+3	47	–1	86	+39	88	+2	81	–7	104	+23	119	+15	126	+7	133	+7	149	+16	131	–18	164	+33	147	–17	169	+22	163	–6	158	–5
7	Heart	11	19	+8	26	+7	46	+20	97	+51	106	+9	132	+26	164	+32	162	–2	179	+17	220	+41	252	32	282	+30	335	+53	249	–86	290	+41	308	+18
8	Pancreas	6	11	+5	9	–2	8	–1	19	+11	14	–5	23	+9	14	–9	19	+5	12	–7	6	–6	6	0	17	+11	10	–7	16	+6	10	–6	10	0
9	Lungs	1	0	–1	0	0	1	+1	1	0	6	+5	5	–1	10	+5	12	+2	14	+2	16	+2	25	+9	25	0	23	–2	9	–14	13	+4	14	+1
10	Heart-lung										2	+2	2	0	1	–1	0	–1	0	0	0	0	0	0	3	+3	2	–1	2	0	2	0	1	–1
11	Small intestine														1	+1	1	0	0	–1	0	0	0	0	0	0	0	0	1	+1	1	0	0	–1
	Total	662	813	+151	942	+129	1060	+118	1363	+303	1307	–56	1345	+38	1400	+55	1522	+122	1485	–37	1704	+219	1896	+192	2193	+297	2427	+234	1960	–467	2318	+358	2555	+237

Table 10

Number of organ transplant recipients in the Russian Federation in 2013–2022

ICD-10 code	Patient count in the Registry (persons)																	
	2013	2014		2015		2016		2017		2018		2019		2020		2021*		2022*	
		Absolute	YoY change (%)	Absolute	YoY change (%)	Absolute	YoY change (%)	Absolute	YoY change (%)	Absolute	YoY change (%)	Absolute	YoY change (%)	Absolute	YoY change (%)	Absolute	YoY change (%)	Absolute	YoY change (%)
Z94.0 Kidney transplant status	6651	7502	12.8	8164	8.8	9063	11.0	9658	6.6	10,851	12.4	11,880	9.5	12,563	5.7	13,059	–	13,721	–
Z94.1 Heart transplant status	416	520	25.0	639	22.9	803	25.7	952	18.6	1164	22.3	1355	16.4	1524	12.5	1725	–	1916	–
Z94.2 Lung transplant status	2	3	50.0	4	33.3	5	25.0	8	60.0	28	250.0	26	–7.1	24	–7.7	–	–	–	–
Z94.4 Liver transplant status	1150	1406	22.3	1649	17.3	1948	18.1	2152	10.5	2632	22.3	3032	15.2	3489	15.1	3902	–	4294	–
Z94.8 Other transplanted organ and tissue status (bone marrow, intestines, pancreas)	334	467	39.8	654	40.0	808	23.5	909	12.5	1135	24.9	1344	18.4	1497	11.4	–	–	–	–
TOTAL	8553	9898	15.7	11,110	12.2	12,627	13.7	13,679	8.3	15,810	15.6	17,637	11.6	19,097	8.3	20,724	8.5	21,969	

* Note. The number of organ transplant recipients is estimated, as it is calculated from the figures of the previous year based on data on the number of organ transplants in 2021, 2022 and data on the average patient survival.

Two deceased-donor liver transplants were performed in Samara Oblast (at Samara State Medical University, Samara);

Three deceased-donor liver transplants were performed in Primorsky Krai (at Primorsky Regional Clinical Hospital No. 1, Vladivostok).

Moscow remains the undisputed leader in the development of organ donation and transplantation in the Russian Federation, demonstrating a high level of donor and transplantation activity in terms of global practice. At the same time, other centers for advanced development of transplant care in the federal subjects of the Russian Federation have been clearly identified in the country, such as the Belyaev Kemerovo Regional Clinical Hospital (Kemerovo), Republican Clinical Hospital (Kazan), Regional Clinical Hospital No. 1 (Tyumen), Irkutsk Regional Clinical Hospital (Irkutsk), Rostov Regional Clinical Hospital (Rostov-on-Don), and the branch of Shumakov Center (Volzhsky).

The number of waitlisted patients at transplant centers remains at approximately the same level, increasing when new centers and organ transplant programs are opened in the federal subjects of the Russian Federation, as well as when transplantation activity at the centers increases. The following are considered as promising tools for effective identification of patients in need and their inclusion in the waiting list of Shumakov Center:

– collaborating with national and regional patient registries;
– interacting with dialysis centers through the compulsory health insurance tariff agreement and, accordingly, the target indicator "number of dialysis patients on the waiting list";
– expanding the indications and increasing the number of telemedicine consultations with Shumakov Center to clarify the indications for organ transplantation and placement on the waiting list;
– activeness and responsibility of the chief freelance nephrologist of the executive authorities of the federal subjects of the Russian Federation in the area of healthcare for related work.

The level of donor activity in Moscow (26.3 p.m.p.), Kemerovo Oblast (15.8 p.m.p.), and the Republic of Tatarstan (13.3 p.m.p.) indicates a high potential for increasing the number of deceased donors in other federal subjects of the Russian Federation with proper organization of this activity, including control by the executive authorities of the federal subjects of the Russian Federation in the field of health care.

The average value for the indicator "proportion of effective brain-dead organ donors" in the Russian Federation is consistently above 90.0%, and above 70.0% for the indicator "proportion of multi-organ donors". This indicates that donor resource utilization in most federal subjects of the Russian Federation involved in medical activities related to organ donation is efficient. Failure to achieve these values in the federal subjects of the Russian Federation (Ryazan Oblast, Tyumen Oblast, Kemerovo Oblast, Samara Oblast) should be considered by managers and health care professionals as an unsatisfactory result of work, and as a basis for developing and implementing a plan of appropriate measures to improve the efficiency of the donor program in the region.

The number of organ transplants in the Russian Federation continues to increase systematically, while the existing capacities of medical organizations, where operations are performed on donors and recipients, make it possible to further increase the volume of transplant care, subject to adequate funding, working with waiting lists and donor support (21 centers perform less than 15 organ transplants per year).

In recent years, the necessary conditions have been created in the Russian Federation to prioritize the provision of transplant care to the pediatric population. All identified children in need of organ transplantation are transplanted as soon as possible, typically at federal centers (Shumakov center, the Russian Children's Clinical Hospital, National Medical Research Center for Children's Health, Petrovsky National Research Centre of Surgery) and a number of regional medical organizations. Further increase in the number of pediatric transplants depends on the efficiency of identifying and routing such patients from the federal subjects of the Russian Federation. The Shumakov Center is constantly interacting with tertiary children's hospitals and with chief freelance pediatricians at executive authorities of the federal subjects of the Russian Federation in the field of health care to address this issue.

In the Russian Federation, all organ recipients are provided with immunosuppressive drugs for life at the expense of the federal budget under the program "14 high-cost nosologies"; a federal registry is in place to implement this program. It is the duty of the health authorities of the federal subjects of the Russian Federation in the field of health care and transplantation centers to provide conditions for regular monitoring of blood immunosuppressant levels in transplanted patients and their counseling by a specialist who has undergone additional training for the management of this patient cohort.

The authors declare no conflict of interest.

REFERENCES

1. Organ donation and transplantation in Russian Federation in 2009. *Transplantology 2009: results and prospects*. Vol. I / Ed. by S.V. Gautier. M.–Tver: Triada, 2010: 8–20.
2. Organ donation and transplantation in Russian Federation in 2006–2010. *Transplantology: results and prospects*. Vol. II. 2010 / Ed. by S.V. Gautier. M.–Tver: Triada, 2011: 18–32.
3. Organ donation and transplantation in Russian Federation in 2011 (IV report of National Registry). *Trans-*

plantology: results and prospects. Vol. III. 2011 / Ed. by S.V. Gautier. M.–Tver: Triada, 2012: 14–37.
4. Organ donation and transplantation in Russian Federation in 2012. (V report of National Registry). *Transplantology: results and prospects*. Vol. IV. 2012 / Ed. by S.V. Gautier. M.–Tver: Triada, 2013: 8–28.
5. Organ donation and transplantation in Russian Federation in 2013 (VI report of National Registry). *Transplantology: results and prospects*. Vol. V. 2013 / Ed. by S.V. Gautier. M.–Tver: Triada, 2014: 32–57.
6. Organ donation and transplantation in Russian Federation in 2014 (VII report of National Registry). *Transplantology: results and prospects*. Vol. VI. 2014 / Ed. by S.V. Gautier. M.–Tver: Triada, 2015: 44–75.
7. Organ donation and transplantation in Russian Federation in 2015 (VIII report of National Registry). *Transplantology: results and prospects*. Vol. VII. 2015 / Ed. by S.V. Gautier. M.–Tver: Triada, 2016: 38–71.
8. Organ donation and transplantation in Russian Federation in 2016 (IX report of National Registry). *Transplantology: results and prospects*. Vol. VIII. 2016 / Ed. by S.V. Gautier. M.–Tver: Triada, 2017: 33–66.
9. Organ donation and transplantation in Russian Federation in 2017 (X report of National Registry). *Transplantology: results and prospects*. Vol. IX. 2017 / Ed. by S.V. Gautier. M.–Tver: Triada, 2018: 26–63.
10. Organ donation and transplantation in Russian Federation in 2018 (XI report of National Registry). *Transplantology: results and prospects*. Vol. X. 2018 / Ed. by S.V. Gautier. M.–Tver: Triada, 2019: 46–85.
11. Organ donation and transplantation in Russian Federation in 2019 (XII report of National Registry). *Transplantology: results and prospects*. Vol. XI. 2019 / Ed. by S.V. Gautier. M.–Tver: Triada, 2020: 52–93.
12. Organ donation and transplantation in Russian Federation in 2020 (XIII report of National Registry). *Transplantology: results and prospects*. Vol. XII. 2020 / Ed. by S.V. Gautier. M.–Tver: Triada, 2021: 22–63.
13. Organ donation and transplantation in Russian Federation in 2021 (XIV report of National Registry). *Transplantology: results and prospects*. Vol. XIII. 2021 / Ed. by S.V. Gautier. M.–Tver: Triada, 2022: 38–72.

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CRYOTECHNOLOGY IN LUNG AND HEART-LUNG TRANSPLANTATION

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Bronchial complications, along with development and progression of chronic dysfunction on the background of chronic rejection, are factors that reduce the quality and life of lung and heart-lung recipients. They also increase the frequency of hospitalizations. Application of cryotechnology is based on the contact effect of extremely low temperatures on organs and tissues using a cryoprobe. This article demonstrates the experience of using cryotechnology in the diagnosis and treatment of complications in lung and heart-lung recipients.

Keywords: lung transplantation, heart-lung transplantation, bronchial complications, cryotechnology, cryoablation, cryobiopsy, cryoadhesion, extraction of airway foreign bodies.

INTRODUCTION

According to the World Health Organisation (WHO) 2019 report, chronic lung diseases, including chronic obstructive pulmonary disease (COPD), are the third leading cause of death worldwide [1]. It is important to note that this report does not take into account COVID-19-associated mortality, whose consequences increased mortality rates significantly due to the complicated course of the disease. Lung transplantation (LT) is currently the only curative treatment for severe chronic respiratory failure [2]. Sixty years have passed since the first LT was performed by J. Hardy et al. Since then, LT has come a long way from single transplantations with a high incidence of adverse outcomes to an almost routine treatment method [3].

Despite improvements in immunosuppressive therapy protocols, surgical techniques, donor organ preservation methods, as well as approaches to early rehabilitation of recipients, the average life expectancy of lung recipients remains at a relatively low level in comparison with recipients of other solid organs, for which there are a number of objective reasons. Based on evaluation of 16,156 consecutive adult LT recipients, Hayanga et al. found that survival rates were reduced in recipients with airway complications (54.6% vs 84.4%, at 1 year, and 33.2% vs 54.2% at five years) [4]. Several authors have reported the incidence of airway complications to be between 2% and 18% [5, 6], and most cases occur in the first year after transplantation. Among them, the greatest number comes from bronchial stenosis (BS), whose incidence varies from 1.4% to 32%. This undoubtedly demonstrates the high interest in methods aimed at early diagnosis and treatment.

At the same time, the main factor limiting the life expectancy of lung recipients is the development and progression of chronic graft dysfunction on the background of chronic rejection [7]. Differential diagnosis of some complications is sometimes difficult due to the similarity of symptoms and lack of pathognomonic signs.

Bronchial stenosis leads to impaired airway patency and decreased respiratory volumes, which, based on the totality of clinical manifestations, can also be interpreted as dysfunction of transplanted lungs. In this case, one of the main methods of differential diagnosis is endoscopic examination.

This article presents the main techniques of endoscopic diagnosis and treatment of airway complications in lung recipients using cryotechnology.

PHYSICAL PRINCIPLES OF CRYOTECHNOLOGY APPLICATION

Cryosurgical techniques used in endoscopic practice are based on local exposure of organs and tissues to low temperatures in the area of contact with the working surface of the cryoprobe. This technique allows for cryobiopsy, cryoablation, cryorecanalization, as well as removal of foreign bodies [8].

The cryoprobe works on the Joule–Thomson physical principle, which consists in changing the temperature of a liquefied gas as a result of a pressure drop from high to atmospheric pressure [9]. Nitrogen oxide (N_2O), carbon dioxide (CO_2), and liquid nitrogen (N_2) are used as liquefied gas. Transition of nitrogen from liquid to gaseous state when it enters from the cryoprobe nozzle is accompanied by a decrease in the temperature of the working part of the tool to –89 °C. Carbon dioxide has

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long been considered unsuitable for use in endoscopy because its expansion forms ice crystals that damage the endoscope. However, modern cryoprobes do not form such crystals, and CO_2 is a good alternative to N_2O, given its lower cost. Liquid nitrogen creates a temperature of –196 °C at the distal end during expansion [8]. That is why this gas has not been widely used due to its greater penetration depth and high risk of perforation.

CRYOBIOPSY IN THE DIFFERENTIAL DIAGNOSIS OF GRAFT REJECTION

Transbronchial lung biopsy (TBB) has become the gold standard for diagnosis of rejection in LT recipients. One of the modern methods is transbronchial lung cryobiopsy (TBLC). As a result of low temperatures, tissues are fixed to the distal edge of the cryoprobe.

Unlike traditional forceps biopsy, the quality of the diagnostic material obtained is much higher when performing TBLC, which is down to the absence of the effect of crushing by the brushes of biopsy forceps [10]. See Fig. 1.

The main complications arising after cryobiopsy include bleeding [11, 12], as well as development of pneumothorax [13, 14].

From September 2019 to April 2022, 13 cryobiopsies were performed in 9 lung transplant recipients at Shumakov National Medical Research Center of Transplantology and Artificial Organs.

Biopsies were performed in the operating room under general anesthesia with the use of high-frequency ventilation and rigid bronchoscope. The video bronchoscope was sequentially inserted through the tube of the rigid bronchoscope into the subsegmental bronchi. A cryoprobe was inserted through the instrument channel of the endoscope. Then, liquefied gas was supplied through the cryoprobe for 3 seconds, which resulted in freezing of the nearby tissues in contact with it. The endoscope with cryoprobe and biopsy specimen was removed from the bronchial tree. The final stage was control bronchoscopy to assess the degree of bleeding and, if necessary, to achieve hemostasis. After the procedure, control chest x-ray was performed to exclude pneumothorax. Biopsy material was fixed in neutral formalin and sent for routine histopathological examination.

The average number of biopsies was 4–5 fragments. The average biopsy size was 12.4 mm^2, which is significantly larger than the average size of the material obtained by forceps biopsy (4.2 mm^2) ($p < 0.05$). The quality of histological preparations of lung biopsy specimens was significantly higher than that obtained by conventional forceps biopsy.

Seven recipients had post-TBLC complications: pneumothorax (3 cases, 2 of which required pleural cavity drainage, 1 was resolved conservatively (Fig. 2)); pulmonary hemorrhage that stopped conservatively (4 cases, 26%). No other complications were recorded.

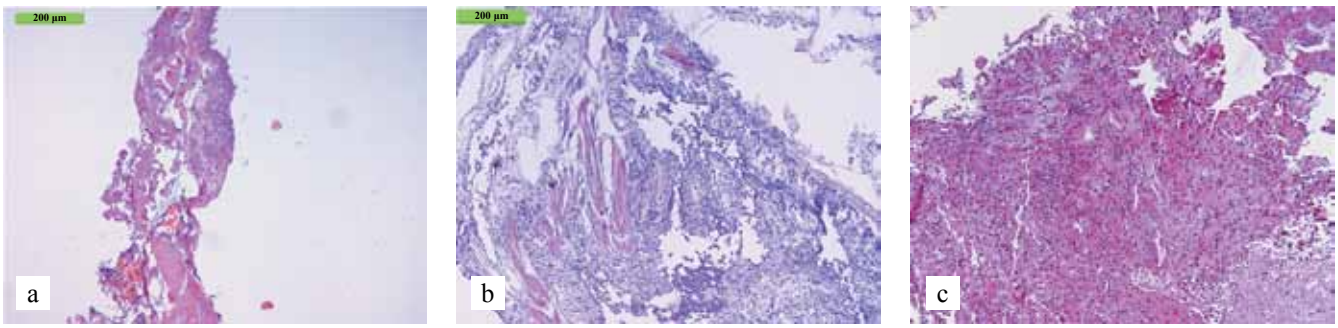

Fig. 1. Dimensions of cryobiopsy specimens: a, standard transbronchial biopsy; b, c, transbronchial lung cryobiopsy

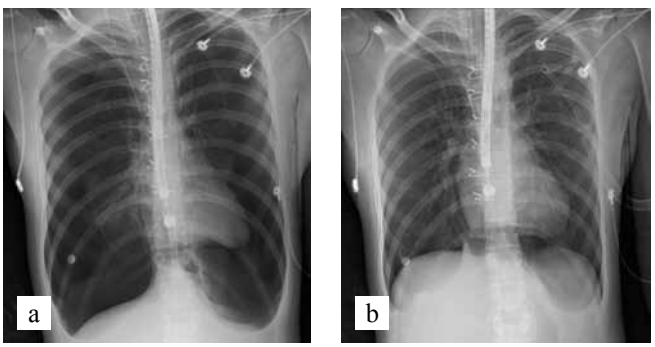

Fig. 2. Chest radiograph: a, bilateral spontaneous pneumothorax in a heart-lung recipient after transbronchial lung cryobiopsy; b, condition after drainage of the thoracic cavity

CRYOABLATION IN THE TREATMENT OF BRONCHIAL STENOSIS

Post-transplant BS is a persistent, breathing-independent narrowing of the lumen due to scar or granulation tissue. The most frequent periods of stenosis occurrence are the first 2–9 months after transplantation [15–19]. Recurrent stenosis of the intermediate bronchus, called vanishing bronchus intermedius syndrome (VBIS) (see Fig. 3), belongs to a separate group. The incidence of this complication is up to 2% [19]. The average life expectancy after diagnosis is up to 25 months [20].

As mentioned above, bronchial stenosis can be divided by mechanisms of occurrence into the following

groups: stenosis caused by the growth of scar tissue [21]; stenosis caused by the growth of granulation tissue.

Cryoablation, also referred to as cryotherapy, involves cycles of rapid freezing (–20 to –100 °C) and slow thawing of tissues, which leads to the formation of intracellular ice crystals and cell death [22–24]. The main mechanisms of intracellular effects are damage to mitochondria and other organelles, cellular dehydration, increased concentration of intracellular electrolytes, and denaturation of membrane lipoproteins. Vascular changes include initial vasoconstriction of arterioles and venules, vascular endothelial damage, decreased intracapillary hydrostatic pressure, and decreased blood flow. It is worth noting that the occurrence of thrombosis of the microvasculature of tissues exposed to low temperatures is the cause of minimal bleeding associated with this method.

It is customary to divide tissues into those that are more sensitive to freeze-induced devitalization, such as skin, mucous membrane, granulation tissue, and tumor cells, and those that are less sensitive, such as fat, cartilage, and connective tissue [25].

The depth of cryotherapy exposure in the bronchial tree is approximately 3 mm, but this depends on the exposure and the gas used [26]. This feature, together with the cartilage's resistance to cryotherapy, reduces the risk of airway perforation. It is important to note that the destructive effects of cryotherapy do not manifest themselves immediately, but are delayed. It takes several days to weeks for tissue necrosis to manifest, during which tissue rejection continues, sometimes requiring removal of necrotic scab during therapeutic bronchoscopy.

M.O. Maiwand et al. used cryoablation as a therapy for granulation stenosis in 21 recipients [27]. Each patient required an average of about 3 cryotherapy sessions. The endoscopic cryotherapy results were rated as excellent or good in 15 patients, and as satisfactory in 6 patients. Eight recipients required endobronchial stenting as part of comprehensive treatment, whereas cryotherapy and balloon dilatation alone were effective in 13 recipients.

Cryoablation complications include bleeding occurring both during the procedure and several days later, mucosal necrosis and bronchial wall perforation, edema, and lumen obstruction by necrotic tissues.

In our practice, cryotherapy was performed in 16 patients with cicatricial granulation stenosis of the intermediate bronchus; a total of 52 cryotherapy sessions were performed. In 30 cases, balloon dilatation was performed as an initial step in order to ensure adequate lumen and preserve ventilation of the distal parts of the lung. Then, three freeze-thaw sessions of 30–45 seconds each were performed using a cryoprobe with a diameter of 2.4 mm. Tissue-freezing time was controlled visually until ice formation on the cryoprobe surface stopped. Tissue thawing was stopped until the cryoprobe moved away from the mucosa. Then, the cryoprobe was moved 5–6 mm away from the affected area, and cryotherapy sessions were repeated until the stenosis area was completely treated (Fig. 4).

Control endoscopic examinations were performed on days 7, 14, and 21 after cryoablation. Twelve patients required repeated cryotherapy sessions. In 7 patients, stenting was performed to preserve the bronchial lumen. No cryotherapy-associated complications were observed.

FOREIGN BODY EXTRACTION

The cryoprobe can be used to extract foreign bodies, mucous and blood clots located in the lumen of the bronchial tree [28, 29].

Some types of foreign bodies, such as staples, metal prostheses, are more difficult to remove from the bronchial tree using a cryoprobe. However, the use of a small amount of sterile sodium chloride solution can improve the effectiveness of this procedure [30].

In our practice, blood clot extraction was performed after pulmonary hemorrhage. Two patients after lung transplantation had pulmonary hemorrhage, for which emergency bronchoscopy was performed. After effective hemostasis, there was still a picture of bronchial obstruction with hemorrhagic clots, cryoextraction was performed using a flexible bronchoscope and cryoprobe (see Fig. 5).

It is worth noting that the removed fragments were less subjected to fragmentation than when classical clot extraction methods were used.

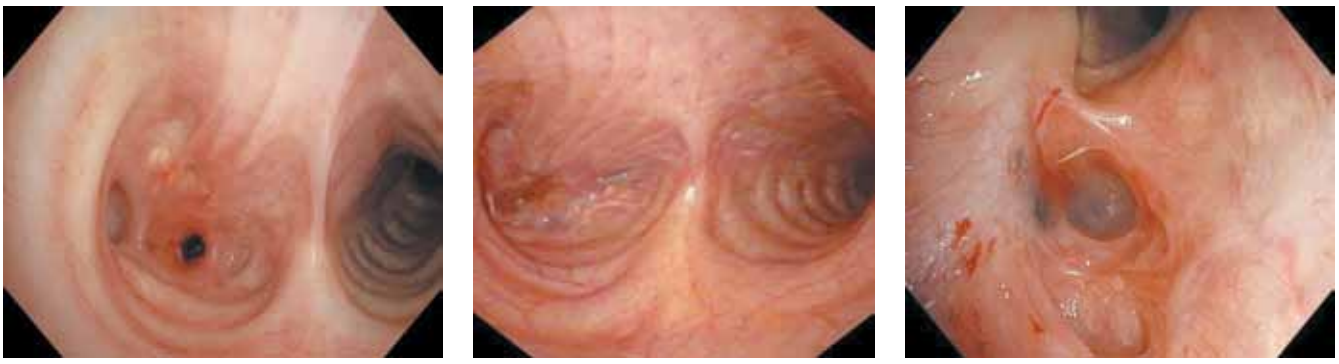

Fig. 3. Variants of intermediate bronchial stenosis

CONCLUSIONS

According to the International Society for Heart and Lung Transplantation (ISHLT), there is a steady trend towards an increase in the total number of lung transplants [7]. However, complications occurring at different times after surgical intervention contribute to the decrease in the quality and duration of life in this patient cohort.

Early diagnosis of complications can improve long-term results after lung and heart-lung transplantation.

TBLC in lung recipients is a highly informative and relatively safe procedure [31, 32]. It can be used to obtain material with greater diagnostic value compared with conventional forceps biopsy. In our study, the incidence of complications is comparable to similar data described in the literature.

It is worth noting that there is no single approach in the treatment of bronchial stenosis [17, 33]. Cryoablation is one of the components of combined treatment in this group of patients [34]. In our practice, no cryotherapy-associated complications were encountered. This suggests that the technique is relatively safe. At the same time, recurrent stenosis after cryoablation makes it necessary to use combined techniques to restore airway patency.

Extraction of foreign bodies, in particular blood clots, using a cryoprobe, is an alternative highly effective method of restoring airway patency. This manipulation significantly reduces the duration of intervention compared to mechanical capture and extraction.

Thus, the use of cryotechnology in endoscopic interventions in lung recipients is a highly effective technique that allows solving a wide range of problems. This has a positive impact on the effectiveness of LT.

The authors declare no conflict of interest.

REFERENCES

1. 10 vedushchikh prichin smerti v mire. *Vsemirnaya organizatsiya zdravookhraneniya: ofitsial'nyy sayt*. https://www.who.int/ru/news-room/fact-sheets/detail/the-top-10-causes-of-death.
2. Transplantologiya i iskusstvennye organy / Pod red. S.V. Gautier. M.: Laboratoriya znaniy, 2018; 319.
3. *Hardy JD, Webb WR, Dalton MR jr and Walker GR*. Lung Homotransplantation in Man: Report of the Initial Case. *JAMA*. 1963 Dec 21; 186: 1065–1074. doi: 10.1001/jama.1963.63710120001010.
4. *Awori Hayanga JW, Aboagye JK, Shigemura N, Hayanga HK, Murphy E, Khaghani A, D'Cunha J*. Airway complications after lung transplantation: Contemporary survival and outcomes. *J Heart Lung Transplant*. 2016 Oct; 35 (10): 1206–1211. doi: 10.1016/j.healun.2016.04.019.
5. *Santacruz JF, Mehta AC*. Airway complications and management after lung transplantation: ischemia, dehiscence, and stenosis. *Proc Am Thorac Soc*. 2009 Jan 15; 6 (1): 79–93. doi: 10.1513/pats.200808-094GO.
6. *Machuzak M, Santacruz JF, Gildea T, Murthy SC*. Airway complications after lung transplantation. *Thorac Surg Clin*. 2015; 25 (1): 55–75. doi: 10.1016/j.thorsurg.2014.09.008.
7. *Khush KK, Cherikh WS, Chambers DC, Harhay MO, Hayes D Jr, Hsich E et al.* The International Thoracic Organ Transplant Registry of the International Society for

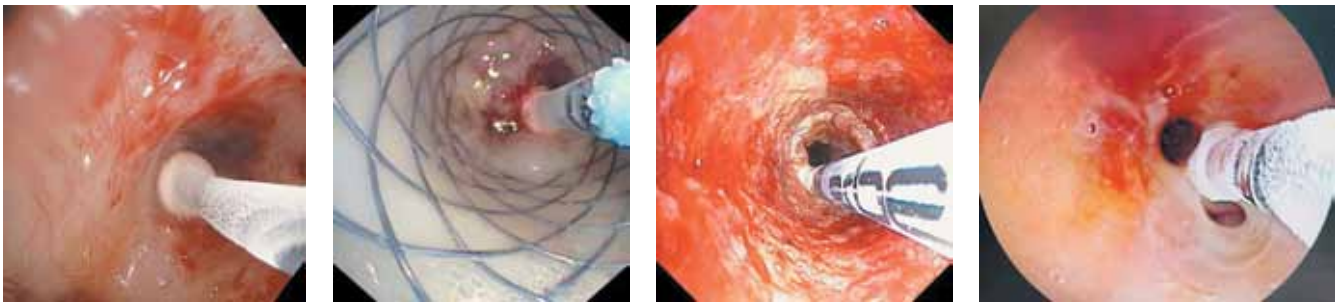

Fig. 4. Cryotherapy with a 1.9 mm and 2.4 mm probe

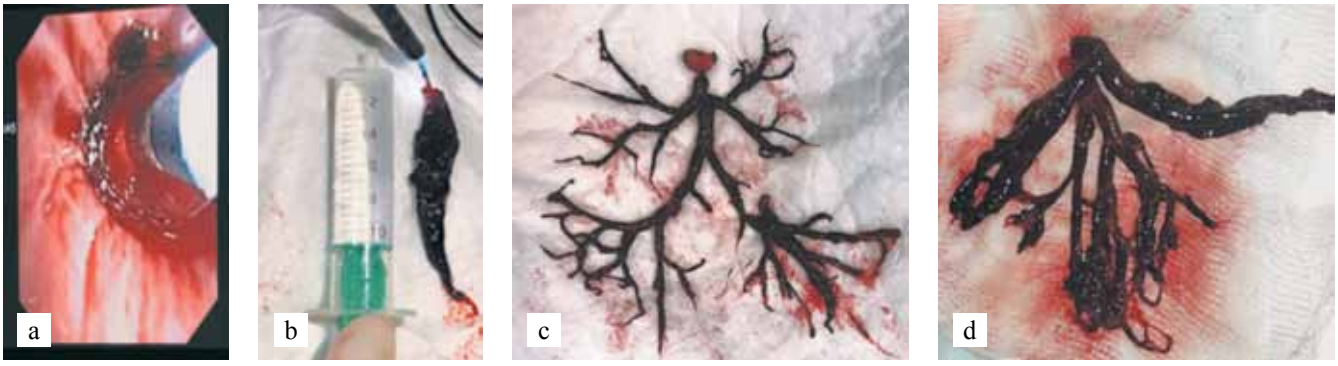

Fig. 5. Clot extraction using a cryoprobe: a, the moment of clots extraction; b–d, extracted clots

Heart and Lung Transplantation: Thirty-sixth adult heart transplantation report – 2019; focus theme: Donor and recipient size match. *J Heart Lung Transplant*. 2019 Oct; 38 (10): 1056–1066. doi: 10.1016/j.healun.2019.08.004.
8. *Noppen M, Meysman M, Van Herreweghe R, Lamote J, D'Haese J, Vincken W.* Bronchoscopic cryotherapy: preliminary experience. *Acta Clin Belg*. 2001 Mar-Apr; 56 (2): 73–77. doi: 10.1179/acb.2001.013.
9. *Homasson JP et al.* Cryotherapy in chest medicine. Springer Science & Business Media, 2012.
10. *Colt HG.* Bronchoscopic cryotechniques in adults. Uptodate, review. 2017. https://www.medilib.ir/uptodate/show/4407.
11. *Herth FJ, Mayer M, Thiboutot J, Kapp CM, Sun J, Zhang X et al*. Safety and Performance of Transbronchial Cryobiopsy for Parenchymal Lung Lesions. *Chest*. 2021 Oct; 160 (4): 1512–1519. doi: 10.1016/j.chest.2021.04.063.
12. *Schumann C, Hetzel J, Babiak AJ, Merk T, Wibmer T, Möller P et al*. Cryoprobe biopsy increases the diagnostic yield in endobronchial tumor lesions. *J Thorac Cardiovasc Surg*. 2010 Aug; 140 (2): 417–421. doi: 10.1016/j.jtcvs.2009.12.028.
13. *Ussavarungsi K, Kern RM, Roden AC, Ryu JH, Edell ES.* Transbronchial Cryobiopsy in Diffuse Parenchymal Lung Disease: Retrospective Analysis of 74 Cases. *Chest*. 2017 Feb; 151 (2): 400–408. doi: 10.1016/j.chest.2016.09.002.
14. *Bango-Álvarez A, Ariza-Prota M, Torres-Rivas H, Fernández-Fernández L, Prieto A, Sánchez I et al*. Transbronchial cryobiopsy in interstitial lung disease: experience in 106 cases - how to do it. *ERJ Open Res*. 2017 Mar 22; 3 (1): 00148-2016. doi: 10.1183/23120541.00148-2016.
15. *Mohanka M, Banga A*. Alterations in Pulmonary Physiology with Lung Transplantation. *Compr Physiol*. 2023 Jan 30; 13 (1): 4269–4293. doi: 10.1002/cphy.c220008.
16. *Lequia L et al.* Post-transplant bronchial stenosis: A single center retrospective study *Chest*. 2022; 162 (4): A2582–A2583.
17. *Kim HH, Jo KW, Shim TS, Ji W, Ahn JH, Oh DK et al*. Incidence, risk factors, and clinical characteristics of airway complications after lung transplantation. *Sci Rep*. 2023 Jan 12; 13 (1): 667. doi: 10.1038/s41598-023-27864-1.
18. *Delbove A, Senage T, Gazengel P, Tissot A, Lacoste P, Cellerin L et al.* Incidence and risk factors of anastomotic complications after lung transplantation. *Ther Adv Respir Dis*. 2022 Jan-Dec; 16: 17534666221110354. doi: 10.1177/17534666221110354.
19. *Santillana EC et al.* A Case Report of Progressive Segmental Stenosis in a Transplant Patient. C44. Case Reports: Pulmonary Potpourri. *Am Thor Soc*. 2022: A4218–A4218.
20. *Marulli G, Loy M, Rizzardi G, Calabrese F, Feltracco P, Sartori F, Rea F.* Surgical treatment of posttransplant bronchial stenoses: case reports. *Transplant Proc.* 2007 Jul-Aug; 39 (6): 1973–1975. doi: 10.1016/j.transproceed.2007.05.021.
21. *Puchalski J, Lee HJ, Sterman DH.* Airway complications following lung transplantation. *Clin Chest Med.* 2011 Jun; 32 (2): 357–366. doi: 10.1016/j.ccm.2011.03.001.
22. *Gage AA, Guest K, Montes M, Caruana JA, Whalen DA Jr.* Effect of varying freezing and thawing rates in experimental cryosurgery. *Cryobiology.* 1985 Apr; 22 (2): 175–182. doi: 10.1016/0011-2240(85)90172-5.
23. *Smith JJ, Fraser J.* An estimation of tissue damage and thermal history in the cryolesion. *Cryobiology.* 1974 Apr; 11 (2): 139–147. doi: 10.1016/0011-2240(74)90303-4.
24. *Miller RH, Mazur P.* Survival of frozen-thawed human red cells as a function of cooling and warming velocities. *Cryobiology.* 1976 Aug; 13 (4): 404–414. doi: 10.1016/0011-2240(76)90096-1.
25. *Rusakov MA.* Endoskopicheskaya kriokhirurgiya trakhei i bronkhov (eksperimental'no-klinicheskoe issledovanie): avtoref. dis. … kand. med. nauk. M., 1983: 18.
26. *Vergnon JM, Huber RM, Moghissi K.* Place of cryotherapy, brachytherapy and photodynamic therapy in therapeutic bronchoscopy of lung cancers. *Eur Respir J.* 2006 Jul; 28 (1): 200–218. doi: 10.1183/09031936.06.00014006.
27. *Maiwand MO, Zehr KJ, Dyke CM, Peralta M, Tadjkarimi S, Khagani A, Yacoub MH.* The role of cryotherapy for airway complications after lung and heart-lung transplantation. *Eur J Cardiothorac Surg.* 1997 Oct; 12 (4): 549–554. doi: 10.1016/s1010-7940(97)00208-x.
28. *Rafanan AL, Mehta AC.* Adult airway foreign body removal. What's new? *Clin Chest Med.* 2001 Jun; 22 (2): 319–330. doi: 10.1016/s0272-5231(05)70046-0.
29. *De Weerdt S, Noppen M, Remels L, Vanherreweghe R, Meysman M, Vincken W.* Successful removal of a massive endobronchial blood clot by means of cryotherapy. *J Bronchol Int Pulmonol*. 2005; 12 (1): 23–24.
30. *Hewlett JC, Rickman OB, Lentz RJ, Prakash UB, Maldonado F.* Foreign body aspiration in adult airways: therapeutic approach. *J Thorac Dis.* 2017 Sep; 9 (9): 3398–3409. doi: 10.21037/jtd.2017.06.137.
31. *Fruchter O, Fridel L, Rosengarten D, Raviv Y, Rosanov V, Kramer MR.* Transbronchial cryo-biopsy in lung transplantation patients: first report. *Respirology.* 2013 May; 18 (4): 669–673. doi: 10.1111/resp.12037.
32. *Tong R, Zhao L, Guo LJ, Zhou GW, Liang CY, Hou G et al.* Application of transbronchial cryobiopsy in the diagnosis of postoperative complications after lung transplantation: a report of 6 cases. *Chin J Tub Resp Dis*. 2023 Jan 12; 46 (1): 34–39. doi: 10.3760/cma.j.cn112147-20220411-00301.
33. *Mahajan AK, Folch E, Khandhar SJ, Channick CL, Santacruz JF, Mehta AC, Nathan SD.* The Diagnosis and Management of Airway Complications Following Lung Transplantation. *Chest.* 2017 Sep; 152 (3): 627–638. doi: 10.1016/j.chest.2017.02.021.
34. *Mathur PN, Wolf KM, Busk MF, Briete WM, Datzman M.* Fiberoptic bronchoscopic cryotherapy in the management of tracheobronchial obstruction. *Chest.* 1996 Sep; 110 (3): 718–723. doi: 10.1378/chest.110.3.718.

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DEVELOPMENT OF GRAFT-VERSUS-HOST DISEASE IN A LIVER RECIPIENT. CLINICAL OBSERVATIONS AND LITERATURE REVIEW

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Graft-versus-host disease (GvHD) after liver transplantation (LT) occurs in 0.2–0.3% of liver transplant recipients. Each case is characterized by individual peculiarities of the clinical picture. There are no standards or clinical guidelines for the treatment of GvHD in solid organ recipients; mortality remains very high among these patients. We present two clinical cases of verified GvHD that developed early after LT, and we offer a brief review of the current state of the art in the study of this problem.

Keywords: liver transplantation, graft-versus-host disease.

INTRODUCTION

GvHD develops in recipients of allogeneic hematopoietic cells and solid organs whose body is unable to reject donor lymphocytes. Clinical manifestations of this response are associated with the fact that donor lymphocytes trigger an immune-mediated reaction against the recipient's antigenically distinct tissues. A distinction is made between cellular and humoral GvHD. Humoral GvHD most often occurs in case of ABO or Rh incompatibility of the donor and recipient and leads only to usually insignificant hemolytic anemia (passenger lymphocyte syndrome) [1]. Cellular GvHD, which we discuss in this paper, is associated with activation and clonal expansion of immunocompetent donor liver lymphocytes with subsequent tissue damage in the recipient. Post-LT cellular GvHD was first described in 1988 [2]. Fortunately, in real clinical practice, there are quite rare GvHD cases occurring following solid organ transplantation. Indirectly, the incident of GvHD in recipients of solid organ transplantation and LT in particular, can be judged from a systematic review published in 2018 by researchers from the Meyo Clinic [3]. In a thorough literature search, up to 2016, the authors found 115 cases of post-transplant GvHD accompanied by dermatologic manifestations. At the same time, the development of solid organ transplant-associated GvHD (SOT GvHD) with dermatologic manifestations was observed in 81 (64.3%) cases. Whereas dermatologic manifestations of GvHD are observed in at least half of the total number of patients with GvHD (the incidence is reported to be up to 92–94% [4, 5]), the number of SOT GvHD cases described so far does not exceed 200. Researchers from the same Clinic recently reported a 0.3% incidence of GvHD (12 cases) in an analysis of all LTs performed between January 1, 2010 and December 31, 2021 (4,585 operations) [6]. In an analysis of the OPTN database, which included 77,416 adult patients who underwent LT between 2003 and 2018, the incidence of fatal GvHD after LT was 0.2% (121) [7].

Our description of cutaneous GvHD in a liver recipient with a review of the state of the art of the study of this problem in 2010 was the first in the national literature [8]. In this paper, we present two clinical cases of verified GvHD that developed early after LT and provide a brief review of the current state of the study of this problem.

Solid organ allografts contain varying numbers of donor leukocytes, which are a mixed population including monocytes, natural killer cells (NK cells), T-cells, and other hematopoietic cells. Transplantation of these immunocompetent cells along with the organ, along with immunosuppressive therapy the recipient receives to prevent rejection, can create conditions for the development

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of tolerance or GvHD. Usually, due to high levels of HLA mismatch, the recipient's immune system destroys the donor lymphocytes. During this time, the donor lymphocyte population in the transplanted organ is replaced by the recipient's lymphoid cells. Less commonly, donor lymphocytes may attack the recipient, causing GvHD.

The theory of GvHD pathogenesis was proposed in 2004 by Taylor et al. [9]. According to this theory, before transplantation, an immunocompromised state that is characterized by increased expression of major tissue compatibility complexes on antigen-presenting cells (APCs) should develop in the recipient's body. In the second step, donor passenger lymphocytes enter the recipient's body and are activated from encountering host APCs. Subsequently, their clones proliferate, mediated by the recipient's interleukin-2 (IL-2). In the final third phase, donor T-cells attack the recipient's tissues, leading to clinical manifestations of the disease [10]. Much evidence confirming the elements of this theory have been accumulated. Thus, an increase in IL-2 level in the vicinity of activated cytotoxic T-cells and accumulation of lymphocytes with donor karyotype in target tissues have been shown [11].

Risk factors for post-LT GvHD include a large age difference between the donor and recipients (the recipient is much older), heterozygosity of the recipient and homozygosity of the donor for the same HLA antigens [12, 13], pre-existing immunosuppression in the recipient, autoimmune diseases, recipient age >65 years, lymphocytopenia before transplantation, cytomegalovirus infection, and multi-organ transplantation [10, 11, 14].

The clinical picture of GvHD in liver recipients is characterized by multi-organ involvement, with the graft being the only organ not involved in the disease. Most commonly, GvHD manifests with fever (identified in 66% of patients [4]), or skin rash or a combination of both. Within a few weeks, the following symptoms of damage to one or more organs and systems are added:

– A rash appears on the skin (in 94% of patients), which becomes confluent and covers all body surfaces, including the palms and soles [4]. Bullae formation and desquamation on large body surfaces is possible [10].
– Gastrointestinal (GI) involvement is most often manifested by diarrhea (in 54% of recipients) [4]. There may be ulceration of the oral mucosa [10] and esophageal ulcerative lesions [15]. In our first observation, there was pronounced gastritis, duodenitis; ileitis and colitis, which may lead to intestinal obstruction [11] or GI bleeding [14] have also been described [14].
– Most recipients develop pancytopenia. Partial variants of hematopoiesis disorders have also been described, such as thrombopenia and leukopenia [16], isolated neutropenia [17].
– A case of isolated lung damage against post-LT GvHD is described. The diagnosis was histologically verified, the authors managed to identify donor cells in the peribronchial space [18].
– There are two descriptions of central nervous system involvement [17, 19]. In one case, development of lymphoproliferative disease (LPD) could not be completely excluded either. Unfortunately, the authors of this case do not provide postmortem data that could confirm or refute the neuro-GvHD version.

Diagnosis of GvHD is based on histological examination of the affected tissue. The diagnosis is most often made on the basis of material from the GI tract [20] or skin biopsy [13, 15]. Immunohistochemical methods can be used for differential diagnosis between different dermatologic diseases [21].

When a donor blood sample is available (usually in liver lobe transplantation from a living donor), a blood test for lymphocyte chimerism (estimation of the percentages of donor lymphocytes to the total number of lymphocytes in the peripheral bloodstream) may be useful in GvHD diagnosis. It should be noted that donor lymphocytes in recipient blood are detected quite frequently. A group of authors from the Meyo Clinic (Rochester, USA) detected these cells in 38 out of 49 recipients 8 weeks after LT [22]. The high incidence of chimerism suggests that it is not the cause of GvHD by itself. There is an assumption that chimerism provides immune tolerance of the recipient and graft. Currently, most researchers distinguish micro- and macrochimerism. The boundary between normal (microchimerism) and pathological (macrochimerism) percentage of donor lymphocytes in the recipient's bloodstream is defined differently by different authors; the proposed variants range from 1 to 10% [10]. Macrochimerism is considered a predictor of GvHD. In some cases, donor lymphocytes may not be detected in the peripheral bloodstream but may be present in target tissues in a GvHD patient [23]. If the donor and recipient are of different genders, it is possible to differentiate lymphocytes in the recipient's tissues by Y-chromosome fish response [14].

To date, there are no standards and clinical guidelines from professional societies for the treatment of GvHD in solid organ recipients. The key issue in the treatment of this patient cohort is the impact on the immune system. Diametrically opposite approaches are discussed: intensification of immunosuppressive therapy or, on the contrary, temporary withdrawal of immunosuppression.

The most common practice in the treatment of GvHD is the use of high-dose glucocorticoids (GCs) (2–10 mg/kg) as first-line therapy. This approach is effective in GvHD patients after bone marrow transplantation [22], but is usually unsuccessful in liver recipients [13]. Attempts to use drugs for induction of immunosuppression directed against T cells have been described. Antithymocyte globulin (ATG) [10, 13, 17, 22], basiliximab or daclizumab [22], and alefacept [17] have been used. Unfortunately, all the described observations ended fatally.

There is a description of the successful use of basiliximab shortly after high-dose methylprednisolone (MP) administration in two patients who developed GvHD symptoms with skin and intestinal lesions 3–5 weeks after LT. The rash disappeared within 3 and 2 weeks, respectively, after basiliximab administration. However, both patients continued to have severe GI symptoms, they developed acute intestinal obstruction, underwent intestinal resection, and only then recovered [11].

Immunosuppression reduction is less frequently used in the treatment of GvHD.

In the literature, there is a report of two patients with relatively mild GvHD, whose symptoms resolved with immunosuppression reduction [24]. In addition, there is a description of successful treatment of GvHD after conversion from tacrolimus to cyclosporine, which was performed due to suspected drug intolerance [14]. In our case (observation 1), such therapeutic tactics were not successful. In a number of GvHD cases, routine maintenance immunosuppression, which should have been prescribed according to the protocol, was canceled, but drugs with multidirectional, including immunosuppressive action (ATG, infliximab, granulocyte colony-stimulating factor, alefacept, MP, interleukin-11, immunoglobulin) were prescribed [10, 16, 17, 22]. Unfortunately, the authors usually did not justify their choice of therapy.

There are suspicions associated with the use of ruxolitinib (a selective JAK inhibitor). Its use in the treatment of GvHD in solid organ recipients is borrowed from the practice of hematologists, for whom the search for effective therapy for glucocorticoid-resistant GvHD after stem cell transplantation is a pressing issue [25]. There is a case in which a patient with advanced GvHD responded to treatment in 10 days, and chimerism regressed after one month of treatment [12].

Two cases of host immune cell infusion have been reported. In an earlier observation, autologous bone marrow transplantation was performed after GvHD was diagnosed using host cells collected before LT, leading to resolution of GvHD [26]. In another case, lymphocytes were harvested from a patient after the development of GvHD and were enriched ex vivo to "transform" into recipient lymphocytes. These cells were then reinfused into the patient, presumably resulting in subsequent recovery of the recipient [27]. In addition, the literature discusses the possibility of liver retransplantation with the aim of eliminating immune aggression from the donor tissues and counting on greater immune tolerance of the new graft.

CLINICAL CASE 1

Patient A., female, 54 years old, was on October 3, 2016, transplanted with the right lobe of the liver of her daughter (28 years old) for cirrhosis as a result of chronic hepatitis C on the background of persistent HCV viremia. Blood group of donor and recipient was I(0), Rh(+). HLA typing: donor A(24), B(48), DRB1(12); recipient A(24), B(38), B(48), DRB1(04), DRB1(12).

Immunosuppression was induced according to standard protocol: 20 mg basiliximab, 1000 mg methylprednisolone (MP) in the liverless period. From day 2, the patient received tacrolimus with target blood concentration of 10–13 ng/mL. The postoperative period was uneventful. She was discharged from the hospital on day 24.

On day 45 after LT, the patient went to a hospital around her place of residence complaining of rash and facial swelling. As a result of glucocorticoid therapy (MP 500 mg IV for 3 days, with continuation of oral prednisolone (PSL) with gradual withdrawal), there was a significant decrease in the rash. On day 59, the patient was hospitalized at the surgical ward of Burnazyan State Medical Research Center with complaints of weakness, maculopapular rash with a tendency to generalization, diarrhea. Examination revealed Coombs-positive hemolytic anemia (erythrocytes 1,350,000/μL), leukopenia (1,400/μL) and thrombocytopenia (61,000/μL). Differential diagnosis was made between acute GvHD, allergic (drug) dermatitis. Immunosuppression was converted to cyclosporine, and drug therapy was minimized. Within 3 days, 1000 mg MP was administered intravenously with subsequent oral administration of PSL at a dose of 125 mg/day. During treatment, an increase in red blood cell count up to 2.5 million/μL and an increase in white blood cell count up to 3,600/μL were noted, diarrhea stopped and skin rash slightly decreased. Allergic dermatitis was diagnosed according to the results of histological examination of skin biopsy. Due to development of diabetes mellitus, a gradual reduction of PSL dose was initiated.

On day 90 after LT, at a PSL dose of 80 mg/day, the rash increased again: papular elements on the background of bright erythema of the skin of the face, chest, abdomen, with involvement of palms and soles (Fig. 1). The situation was considered as resistance to GC. To overcome this, a repeated course of therapy with 1000 mg MP was carried out for 5 days, PSL oral dose was increased to 100 mg/day, biopsy of the changed skin was repeatedly performed: GvHD was verified. Linear chimerism study revealed 9.8% of donor leukocytes in the recipient's peripheral blood. Bone marrow study did not reveal myelopoiesis suppression. Despite the slight positive dynamics in the patient's condition, GCs therapy did not lead to GvHD resolution. PSL reduction was initiated, mycophenolic acid (MFA, 2160 mg/day) was added, and basiliximab (20 mg) was administered twice. The MFA dose was selected taking into account hypoalbuminemia (21 g/L), concomitant therapy with high-dose cyclosporine (400 mg/day), and guided by doses used by hematologists in the treatment of acute GvHD after bone marrow transplantation. Shortly after starting MFA, the rash turned pale, and by day 134 after

GvHD, the skin rashes had completely cleared (Fig. 2). Despite successful treatment of skin manifestations, the patient remained anemic and developed nephrotic syndrome (histologically, membranous nephropathy).

The patient was discharged from the hospital on day 158 after LT, 113 days after the appearance of the first cutaneous manifestations of GvHD. At discharge, the patient's condition was satisfactory, rash regressed completely, and signs of secondary Cushing's syndrome were observed. Steroid-induced diabetes and high blood pressure were compensated.

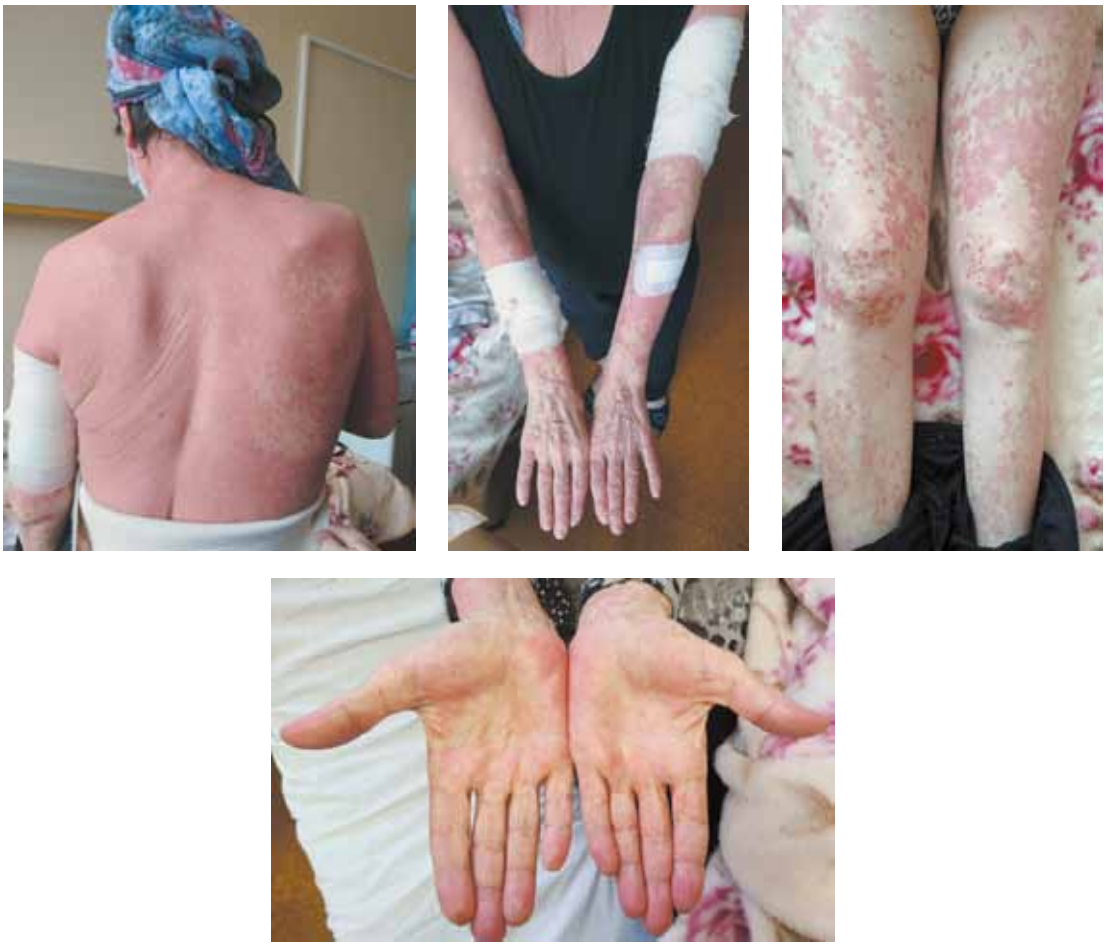

Fig. 1. Clinical case 1. Cutaneous manifestations

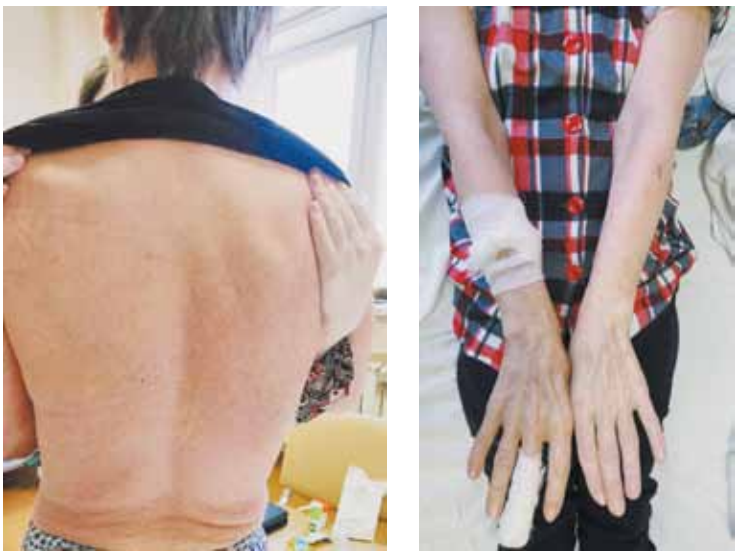

Fig. 2. Clinical case 1. Dynamics of cutaneous manifestations

Four weeks later, on April 10, 2017, the patient was hospitalized again at Burnazyan State Medical Research Center in Moscow with complaints of severe weakness, diarrhea, resistant to treatment, lack of appetite, and resumption of skin rash. Hypoalbuminemia (31 g/L), anemia (hemoglobin 100 g/L), leukopenia 3.1 × 10^9/L with normal platelet levels were observed.

During the first days of hospitalization, dysphagia appeared and increased. Gastroscopy showed that the gastric walls were sharply edematous, there was contact bleeding, and microabscess formation was noted. Peristalsis could not be traced. Biopsy was not taken because of the high risk of bleeding. Progression of GvHD with GI involvement was suspected.

There was an attempt at ATG therapy at a dose of 10 mg/kg. After two administrations, diarrhea decreased, dysphagia was relieved, and skin rash regressed. At the same time, due to adverse events – leukopenia, thrombocytopenia, general weakness – the drug dose was reduced to 5 mg/kg at the third administration, and then ATG infusion was stopped due to increasing weakness. On day 205 after LT, aspiration of gastric contents occurred, which resulted in the development of severe multisegmental pneumonia. Death came the next day.

Autopsy revealed moderate ascites, hydrothorax, multiple hemorrhages, and graft hypertrophy. Particularly severe was digestive tract lesion, whose symptoms were determining the severity of the patient's condition for a relatively short time. On examination of the stomach, most of the mucosa was found to be intact, the folds were flattened. There were pinpoint hemorrhages all over the surface, defects up to 5 mm in diameter along the posterior wall of the body (Fig. 3). The small intestine mucosa was with numerous rounded superficial erosions (0.3 to 0.7 cm in diameter) (Fig. 4). The large intestine had numerous ulcers and circular banded hemorrhages in the mucosa, there were no macroscopic intact mucosal areas.

CLINICAL CASE 2

Patient V., born in 1961, was admitted at the surgical ward of the Center for Surgery and Donor Coordination (CSDC), Rostov Regional Clinical Hospital on November 27, 2022, with complaints of general weakness, jaundice, increased abdominal volume, no effect

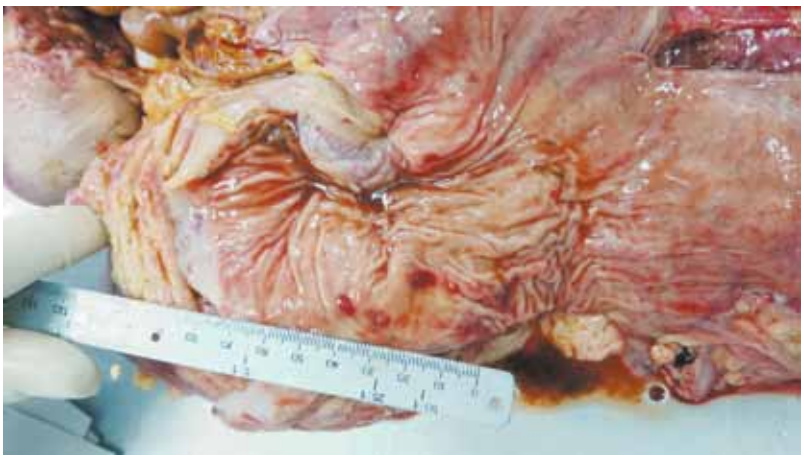

Fig. 3. Clinical case 1. Stomach. Autopsy

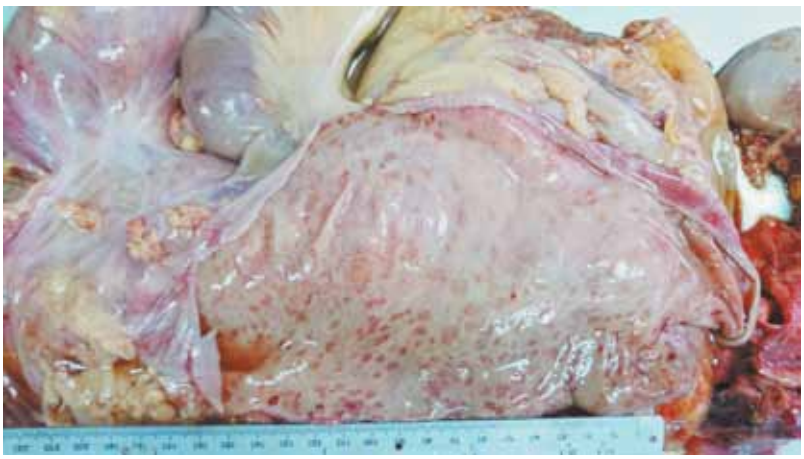

Fig. 4. Clinical case 1. Small intestinal mucosa. Autopsy

of diuretics, dyspnea on exertion, leg swelling, impaired attention and sleep, periodic loss of orientation in time and space. She considers herself a patient since 2013, when cirrhosis of mixed etiology (hepatitis C virus and alcohol) was first diagnosed. Antiviral therapy was not performed; she continued to take alcohol. Signs of decompensation – ascitic-edematous syndrome, jaundice, and hepatic encephalopathy – started appearing from 2020. In October 2022, she was placed on the LT waitlist.

On November 27, 2022, orthotopic LT from a single-group deceased donor was performed. Introductory immunosuppression with basiliximab and methylprednisolone was administered according to the standard protocol. The early postoperative period was uneventful. Given the presence of HCV RNA in the blood, MP was canceled after 7 days, maintenance immunosuppression was limited to extended-release tacrolimus (3.5 mg/day, tacrolimus concentration from 4.5 to 13.0 ng/mL).

She was transferred to the surgical ward on day 7. Anaemia (haemoglobin 92 g/L, erythrocytes 3.4 × 10^{12}/L, hematocrit 25%), leukopenia (3.0 × 10^{9}/L), thrombocytopenia (93.0 × 10^{9}/L) were observed. Functional liver test indicators and creatinine levels remained within normal values.

On day 11 after LT, creatinine increased to 343 μmol/L and urea to 45 mmol/L. The estimated glomerular filtration rate, eGFR, (CKD-EPI) was 12 mL/min/1.73 m^2, diuresis was 1600 mL/day.

Nausea, weakness, tremor, and ascites gradually increased. Conversion of immunosuppressive therapy was performed: tacrolimus was canceled, everolimus was prescribed (blood concentration 7.3–8.9 ng/mL). On day 17 after LT, ultrasound revealed increased linear blood flow rate up to 360 cm/s in the projection of hepatic artery anastomosis. Selective hepatic angiography revealed an arterial anastomosis stenosis up to 80%. Therefore, 3 BioMime intravascular stents were implanted with subsequent balloon catheter dilation. Control angiography showed that the hepatic artery was patent, no residual restenosis was detected. Abdominal cavity drainage was also performed – ascitic fluid without impurities and no microflora growth was obtained.

On day 18 after LT, remittent fever, low blood pressure, intractable nausea and vomiting, diarrhea up to 12 times a day, thrombocytopenia increased. Extracorporeal hemocorrection procedures (veno-venous hemodiafiltration sessions, plasma collection) were performed, renal function was normalized. On day 24 after LT, rashes appeared on the patient's neck, upper and lower limbs in the form of petechial elements, confluent erythematous patches up to 7–8 cm in diameter (Fig. 5). Thrombocytopenia increased (26 × 10^{9}/L), agranulocytosis developed (0.1 × 10^{9}/L). Kidney dysfunction persisted (creatinine, 174 μmol/L; eGFR, 27 ml/min/1.73 m^2). Alanine aminotransferase and aspartate aminotransferase activity was slightly increased (less than 2 times the upper limits of the norm).

Given the presence of fever, rash, diarrhea, and severe cytopenia, we assumed that the patient had GvHD. Differential diagnosis was made with sepsis, including fungal sepsis (repeated blood cultures for mycoses). Antibacterial (tigecycline, ceftazidime with avibactam, cefepime with sulbactam) and antifungal therapy (anidulafungin) was performed. Despite the therapy, the patient's condition did not improve, dermatologic manifestations progressed, which is not typical for fungal and bacterial sepsis, therefore, we decided to take a skin flap for histological examination.

The results obtained show phenomena of dyskeratosis, parakeratosis and hyperkeratosis (Fig. 6). In the

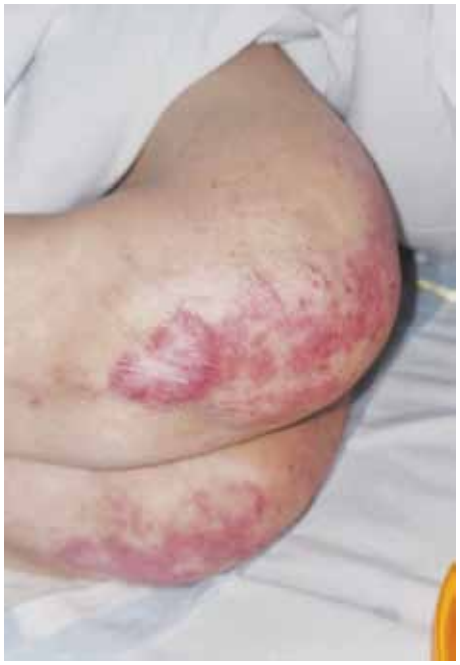

Fig. 5. Clinical case 2. Cutaneous manifestations

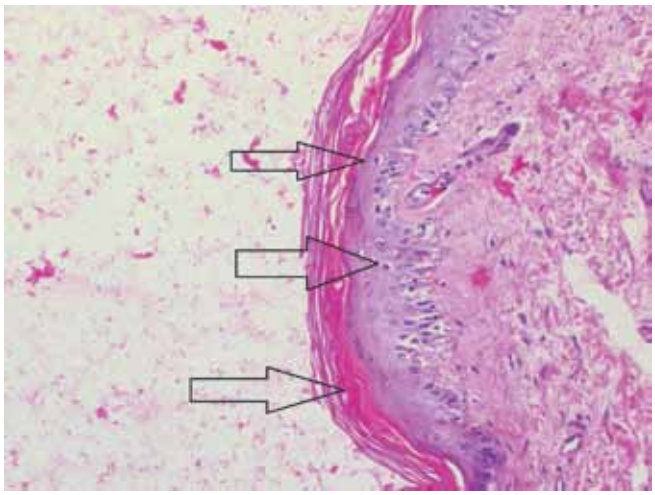

Fig. 6. Clinical case 2. Histologic examination of skin flap. Dyskeratosis, parakeratosis and hyperkeratosis phenomena

basal layer of epidermis, there was pronounced vacuolization of epitheliocytes with focal formation of slits at the border with the dermis (Fig. 7); in the adjacent dermis there was lymphoma-infiltrating macrophages with tropism to the basal layer of epidermis. According to Coons' immunohistochemistry: CD3 fixation was detected in the lymphoid infiltrate of the upper layers of the dermis and basal layer of the epidermis. Fixation of IgG, IgM, CD20 was not detected (Fig. 8). The histological picture is characteristic of grade 2–3 GvHD. Esophagogastroduodenoscopy (EGD test) shows that the mucosa of the duodenal bulb was markedly edematous, covered with whitish plaque (Fig. 9).

Histological examination showed fragments of the duodenal mucosa with pronounced lymphoid infiltration of the intrinsic fibrous lamina on the border with the epithelium, erosions on the epithelium surface and extensive spore clusters of microscopic fungus, morphologically similar to Candida fungi (Fig. 10). The histologic picture is characteristic of GvHD; there was widespread fungal lesion of the duodenal mucosa. GvHD with skin lesions, invasive candidiasis was diagnosed. On December 12, 2022, bacteriological blood test results were obtained: growth of Candida glabrata was detected, caspofungin was added to the therapy.

The patient received parenteral nutrition. Immunosuppression with everolimus (1 mg/day) was continued. The patient's condition worsened, febrile fever and dyspeptic syndrome, pancytopenia persisted. Filgrastim was prescribed, transfusions of thromboconcentrate and fresh frozen plasma were performed.

On day 25 after LT, guided by the escalating skin manifestations, the results of histological examination of the skin flap and literature data on the use of high-dose GCs as first-line therapy in the treatment of GvHD, the team decided to perform pulse therapy (intravenous pulses of 1000 mg methylprednisolone) for three days under the cover of antibacterial and antifungal reserve drugs (polymyxin B, caspofungin). The patient's condition remained extremely severe with increasing multi-organ failure, anemia, hemorrhagic syndrome, and psychomotor agitation. The patient died on December 28, 2022, day 32 after LT.

At autopsy, the mucosa of the esophagus, stomach and small intestine was flattened, thin, red-brown in color, with dotted and spotty hemorrhages 0.3–0.8 cm in diameter. On the section, the colon wall layers were indistinguishable. According to histological examination, there were areas of esophageal mucosa ulceration,

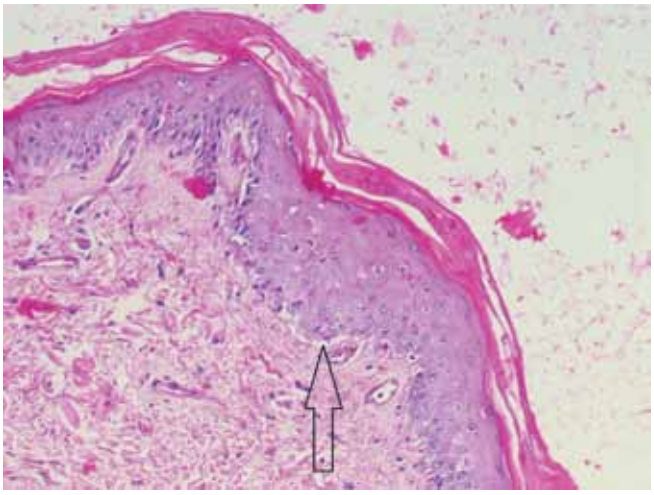

Fig. 7. Clinical case 2. Histological examination of skin flap. Vacuolization of epithelial cells with focal formation of gaps at the border with the dermis

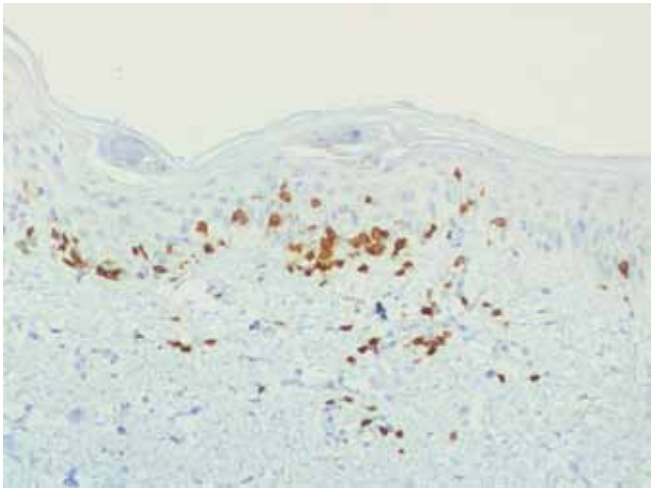

Fig. 8. Clinical case 2. Immunohistochemistry (IHC) analysis by Albert Coons method: CD3 fixation was detected in the lymphoid infiltrate of the upper layers of the dermis and basal layer of the epidermis

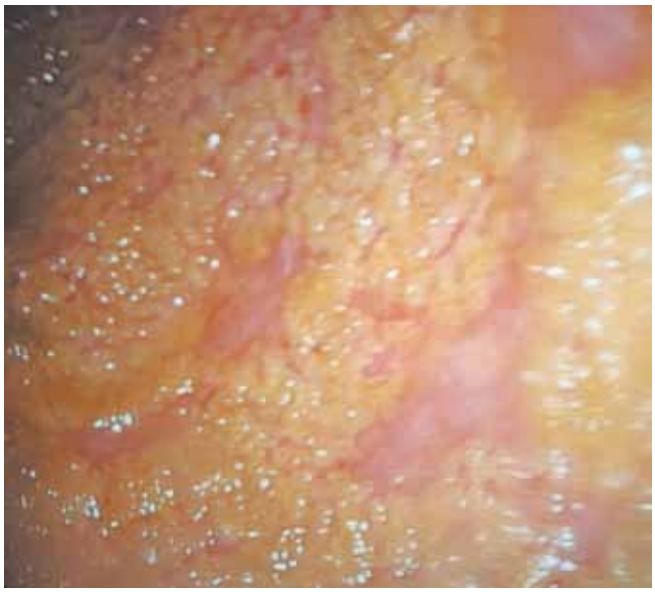

Fig. 9. Clinical case 2. Mucosa of the duodenal bulb. Esophagogastroduodenoscopy (EGD test) data

subtotal desquamation of the mucosa of the small and large intestine with extensive hemorrhages, leukocytic infiltration and multiple accumulations of blastospores of microscopic fungus. The liver graft was edematous, plethoric, the anastomoses were consistent (Fig. 11). Microscopically, there were multiple microabscesses with leukocytic infiltration and accumulation of mycotic flora (Fig. 12).

The bone marrow was sharply hypocellular, represented by maturing forms of granulocytic sprout, with sharp hypoplasia of erythroid and megakaryocytic sprouts.

Pathologists confirmed the clinical diagnosis of GvHD, an acute form with skin and GI mucosa lesions. The course of the underlying disease was complicated by septicopyemia caused by mycotic microflora (Candida glabrata). Subtotal necrosis of the epithelium of convoluted tubules was detected in the kidneys, which, together with pulmonary and cerebral edema, was the immediate cause of the patient's death.

DISCUSSION

We have cited two clinical cases of a cellular modulation of acute GvHD that developed early after LT. It should be noted that these observations are very rare (one in each LT center over decades of clinical practice). The last review that is known to us, which addresses this issue dates back to 2012, featuring 87 patients [4]. To date (according to our estimates), there are no more than 200 descriptions of this pathology in world literature.

Both of our patients had the main clinical manifestations of GvHD (fever, typical rash, diarrhea, pancytopenia). Diagnosis in both cases was confirmed histo-

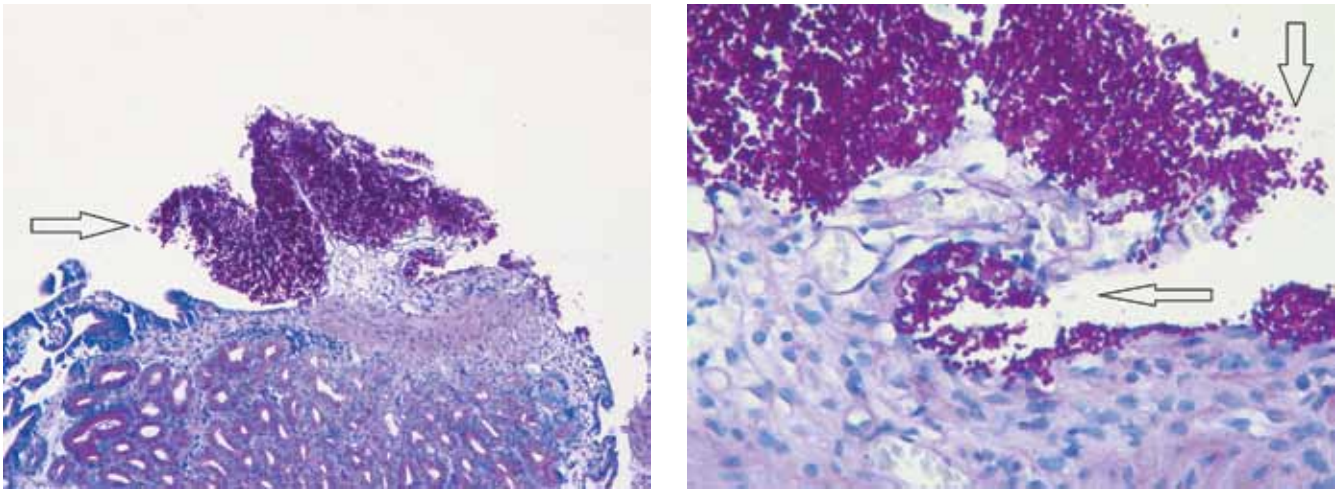

Fig. 10. Clinical case 2. Duodenal mucosal fragments with marked lymphoid infiltration of the intrinsic fibrous lamina at the border with the epithelium, erosions on the epithelial surface and extensive accumulations of spores of microscopic fungus

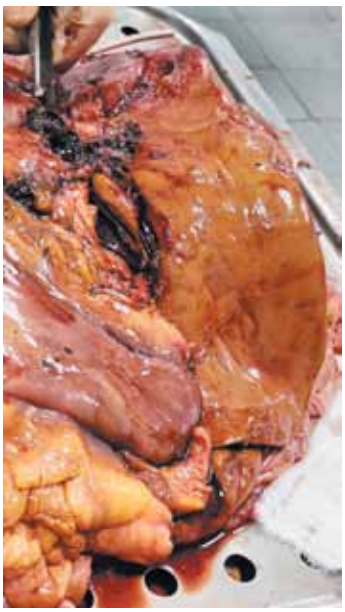

Fig. 11. Clinical case 2. Liver. Autopsy

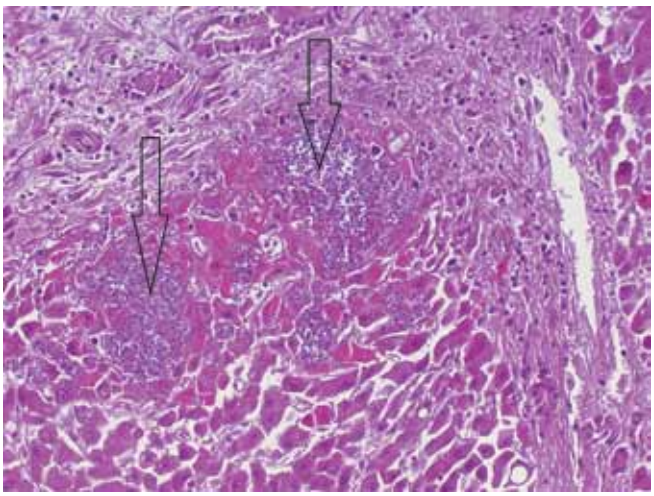

Fig. 12. Clinical case 2. Accumulations of blastospores of microscopic fungus in the liver tissue. 400× magnification, H&E stain

logically. Characteristic histological features of GvHD, detected during skin examination, include basal vacuolar changes, dyskeratosis, apoptosis, lymphocytic infiltration, and in severe cases, subepidermal cleft formation. On the oral mucosa, there were ulcerations, dyskeratotic epithelium with atypia, acute and chronic inflammatory infiltrates in the intrinsic lamina. In the GI mucosa, apoptosis of epithelium or crypts, destruction of glands, and lymphocytic infiltration were detected [28].

In the first case, we had access to donor and recipient HLA studies. Our patient's donor was homozygous for the three alleles for which the recipient was heterozygous,. This is considered to be one of the most significant risk factors of GvHD [29]. Since in the second case, LT was performed from a deceased donor, such a study was not available.

MFA drugs have been successfully used by hematologists to prevent and treat GvHD that complicates bone marrow transplantation. We have not been able to find any experience with MFA for the treatment of GvHD after solid organ transplantation. Administration of MFA in our first patient resulted in a temporary success with regression of dermatologic manifestations. MFA dose was calculated considering drug interactions with cyclosporine and peculiarities of pharmacokinetics in patients with hypoalbuminemia [30]. At the same time, the use of MFA in GvHD patients should be treated with caution because of the risk of drug-induced colitis, which may occur under the guise of GvHD-associated colitis [31, 32].

Unfortunately, despite the therapy, both patients died.

The prognosis of solid organ recipients with GvHD remains unsatisfactory. Mortality exceeds 75–85% [5, 9, 22]. The main causes of death in these patients include infectious complications, which, on the background of deep neutropenia, become septic in nature. For instance, 10 (83.3%) out of 12 liver recipients with acute GvHD observed at the Meyo Clinic (Rochester, USA) developed severe infections that resulted in death [6]. Nosocomial bacteremia caused by intestinal bacteria such as vancomycin-resistant enterococci and gram-negative bacilli was the most common. Invasive fungal infections, cytomegalovirus reactivation, and colitis caused by clostridial flora, have also been reported. The authors suggest that treatment strategies should be determined based on the degree of neutropenia – inhaled levofloxacin and pentamidine for prophylaxis of pneumocystis pneumonia, posaconazole for prophylaxis of invasive mycoses, and valganciclovir. Other causes of death in patients with GvHD include hemorrhage and multi-organ failure.

CONCLUSION

Despite the rarity of post-organ transplant GvHD, its mortality rates are high, and therapy has not been developed. Diametrically opposite approaches have been proposed, such as increasing immunosuppression or decreasing it up to complete cancellation. The experience of treatment of GvHD after bone marrow transplantation cannot be mechanically transferred to solid organ recipients, which is confirmed by our cases. The descriptions of patients with post-LT GvHD, which are available in the world literature, need to be generalized and analyzed, both in terms of risk factor identification, early diagnosis, and optimization of treatment protocols. Infectious complications are the main causes of death in liver transplant recipients who develop GvHD. Therefore, increased prophylaxis for suspected GvHD, followed by an intensified immunosuppression protocol, is necessary. We believe it is important to perform early upper and lower GI endoscopy in solid organ recipients with suspected GvHD. These examinations will allow to detect GI lesions before the development of clinical manifestations, and possibly reevaluate the severity and prognosis of the disease.

The aim of this publication is to sensitize physicians on the problem of GvHD after solid organ transplantation in the hope of reducing mortality. To this end, it is important to be alert to the diagnosis of GvHD and to initiate treatment early enough. The authors recognize the lack of scientific validity of conclusions that are based on descriptions of individual cases or case series. However, in a rare disease such as GvHD after solid organ transplantation, individual cases are the best data we have. Physicians should report any experience with GvHD treatment.

The authors declare no conflict of interest.

REFERENCES

1. *Sushkov AI, Malinovskaya YuO, Miloserdov IA, Moysyuk YaG.* Severe immune hemolytic anemia after liver transplantation. *Russian Journal of Transplantology and Artificial Organs*. 2013; 15 (2): 109–112. (In Russ.). doi: 10.15825/1995-1191-2013-2-109-112.
2. *Burdick JF, Vogelsang GB, Smith WJ, Farmer ER, Bias WB, Kaufmann SH et al*. Severe graft-versus-host disease in a liver-transplant recipient. *N Engl J Med.* 1988 Mar 17; 318 (11): 689–691.
3. *Kim GY, Schmelkin LA, Davis MDP*, *El-Azhary RA, Farrell AM, Meves A, Lehman JS.* Dermatologic manifestations of solid organ transplantation-associated graft-versus-host disease: A systematic review. *J Am Acad Dermatol*. 2018; 78 (6): 1097–1101e1.
4. *Akbulut S, Yilmaz M, Yilmaz S.* Graft-versus-host disease after liver transplantation: A comprehensive literature review. *World J Gastroenterol*. 2012; 18 (37): 5240–5248.
5. *Murali AR, Chandra S, Stewart Z, Blazar BR, Farooq U, Ince MN, Dunkelberg J.* Graft Versus Host Disease After Liver Transplantation in Adults: A Case series, Review of Literature, and an Approach to Management. *Transplantation*. 2016; 100 (12): 2661–2670.
6. *Chesdachai S, Udompap P, Yetmar ZA, Watt KD, Aqel BA, Yang L, Beam E.* Infectious complications in

acute graft-versus-host disease after liver transplantation. *Transpl Infect Dis*. 2022; 24 (4): e13843.
7. *Kitajima T, Henry M, Ivanics T, Yeddula S, Collins K, Rizzari M et al.* Incidence and Risk Factors for Fatal Graft-versus-host Disease After Liver Transplantation. *Transplantation.* 2021; 105 (12): 2571–2578.
8. *Skvortsova YuV, Olisov OD, Syutkin VE, Konovalov DM, Chzhao AV.* Cutaneous graft-versus-host reaction in a female patient a year after orthotopic liver transplantation: a case report and a review of literature. *Transplantologiya*. 2010; 1: 61–67. (In Russ.). doi: 10.23873/2074-0506-2010-0-1-61-67.
9. *Taylor AL, Gibbs P, Bradley JA.* Acute graft versus host disease following liver transplantation: the enemy within. *Am J Transplant.* 2004; 4 (4): 466–474.
10. *Tian M, Lyu Y, Wang B, Liu C, Yu L, Shi JH et al.* Diagnosis and treatment of acute graft-versus-host disease after liver transplantation: Report of six cases. *World J Clin Cases.* 2021; 9 (30): 9255–9268.
11. *Sudhindran S, Taylor A, Delriviere L, Collins VP, Liu L, Taylor CJ et al.* Treatment of graft-versus-host disease after liver transplantation with basiliximab followed by bowel resection. *Am J Transplant.* 2003; 3 (8): 1024–1029.
12. *Jacobs MT, Olson M, Ferreira BP, Jin R, Hachem R, Byers D et al.* The use of ruxolitinib for acute graft-versus-host disease developing after solid organ transplantation. *Am J Transplant.* 2020; 20 (2): 589–592.
13. *Auerbach JS, Schott CK.* Solid-Organ Graft-Versus-Host Disease After Liver Transplant*:* A Case Report. *Crit Care Nurse.* 2016; 36 (3): e7–e11.
14. *Elsiesy H, Ibrahim A, Selim K, Al Sebayel M, Broering D, Al Hamoudi W et al.* Graft-versus-Host Disease after Liver Transplantation: A Single-Center Case Series. *Ann Transplant*. 2015; 20: 397–401.
15. *Ofosu A, Zabolotsky A, Rufail M, Spataro J, Civan J.* Graft-versus-host disease, a rare complication after orthotopic liver transplantation. *Clin Case Rep.* 2018; 6 (1): 238–239.
16. *Qiu W, Lv GY, Jiang C, Zhang P, Sun XD, Shi XJ et al.* Successful salvage treatment of acute graft-versus-host disease after liver transplantation by withdrawal of immunosuppression: a case report. *Korean J Hepatobiliary Pancreat Surg.* 2016; 20 (1): 38–43.
17. *Rogulj IM, Deeg J, Lee SJ.* Acute graft versus host disease after orthotopic liver transplantation. *J Hematol Oncol*. 2012; 5: 50.
18. *Knox KS, Behnia M, Smith LR, Vance GH, Busk M, Cummings OW et al.* Acute graft-versus-host disease of the lung after liver transplantation. *Liver Transpl*. 2002; 8 (10): 968–971.
19. *Massey V, Martel V, Nguyen DK, Busque L, Chapdelaine H, Keezer MR.* Acute central nervous system graft-versus-host-disease after liver transplantation. *Transpl Immunol.* 2022; 70: 101521.
20. *Kreft A, Hippe K, Wagner-Drouet EM, Ries I, Kandulski A, Büttner-Herold M et al.* An investigation of the diagnostic, predictive, and prognostic impacts of three colonic biopsy grading systems for acute graft versus host disease. *PloS One.* 2021; 16 (8): e0256543.
21. *Wegner J, Weidenthaler-Barth B, Engelbert J, Knothe M, Braun C, Helbig D et al.* Immunohistochemical markers for histopathological diagnosis and differentiation of acute cutaneous graft-versus-host disease. *Exp Dermatol*. 2021; 30 (12): 1814–1819.
22. *Perri R, Assi M, Talwalkar J, Heimbach J, Hogan W, Moore SB, Rosen CB.* Graft *vs* host disease after liver transplantation: a new approach is needed. *Liver Transpl.* 2007; 13 (8): 1092–1099.
23. *Schulman JM, Yoon C, Schwarz J, Vagefi PA, Mully TW, Shinkai K.* Absence of peripheral blood chimerism in graft-vs-host disease following orthotopic liver transplantation: case report and review of the literature. *Int J Dermatol.* 2014; 53 (11): e492–498.
24. *Chinnakotla S, Smith DM, Domiati-Saad R, Agura ED, Watkins DL, Netto G et al.* Acute graft-versus-host disease after liver transplantation: role of withdrawal of immunosuppression in therapeutic management. *Liver Transpl.* 2007; 13 (1): 157–161.
25. *Zhang MY, Zhao P, Zhang Y, Wang JS.* Efficacy and safety of ruxolitinib for steroid-refractory graft-versus-host disease: Systematic review and meta-analysis of randomised and non-randomised studies. *PloS One*. 2022; 17 (7): e0271979.
26. *Ricordi C, Tzakis AG, Zeevi A, Rybka WB, Demetris AJ, Fontes PA et al.* Reversal of graft-versus-host disease with infusion of autologous bone marrow. *Cell Transplant*. 1994; 3 (2): 187–192.
27. *Kuball J, Theobald M, Ferreira EA, Hess G, Burg J, Maccagno G et al.* Control of organ transplant-associated graft-versus-host disease by activated host lymphocyte infusions. *Transplantation*. 2004; 78 (12): 1774–1779.
28. *Cooper JP, Abkowitz JL.* How I diagnose and treat acute graft-versus-host disease after solid organ transplantation. *Blood*. 2023 Mar 9; 141 (10): 1136–1146.
29. *Kim SJ, Park S, Rhu J, Kim JM, Choi GS, Joh JW.* The clinical impact of donor against recipient HLA one way mismatch on the occurrence of graft versus host disease in liver transplantation. *Sci Rep*. 2022; 12 (1): 20337.
30. *Hiwarkar P, Shaw BE, Tredger JM, Brown NW, Kulkarni S, Saso R et al.* Mycophenolic acid trough level monitoring: relevance in acute and chronic graft versus host disease and its relation with albumin. *Clin Transplant*. 2011; 25 (2): 222–227.
31. *Patra S, Vij M, Sukanya B, Kapoor D.* Mycophenolate mofetil-induced colitis with graft versus host disease-like features in a liver transplant recipient. *Indian J Pathol Microbiol*. 2012; 55 (4): 506–508.
32. *Papadimitriou JC, Cangro CB, Lustberg A, Khaled A, Nogueira J, Wiland A et al.* Histologic features of mycophenolate mofetil-related colitis: a graft-versus-host disease-like pattern. *Int J Surg Pathol*. 2003; 11 (4): 295–302.

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PHYSICAL REHABILITATION IN PEDIATRIC ORGAN RECIPIENTS

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The paper analyzes the literature on physical rehabilitation in transplantology. The medical and social aspects of rehabilitation and peculiarities of physical rehabilitation in child organ recipients are reflected. A rise in the number of organ recipients, including children, is noted. The role of physical rehabilitation in increasing the body's adaptive reserves at the pre- and postoperative stage and improving the quality of life is described.

Keywords: transplantology, children, organ donation, physical rehabilitation, exercise, rehabilitation, quality of life.

INTRODUCTION

Transplantology, along with genetic engineering and reproductive medicine, is one of the most important areas of development of life-saving high technologies. Basic explantation and transplantation surgery techniques have led to significant advances in overcoming the problem of tissue compatibility, patients who were previously considered irredeemable can now be treated.

The 2024 strategy for the development of transplant care in the Russian Federation is defined in the departmental target program "*Organ Donation and Transplantation in the Russian Federation*", approved by the Russian Ministry of Health (Resolution No. 365 of June 4, 2019).

The goal of the departmental target program is to increase the availability of medical care by organ transplantation to 25.2 per million population by 2024 [1].

According to statistical data, there is an increase in patients with transplanted organs in the Russian Federation (Table 1) [2].

There is an obvious positive trend in the provision of pediatric transplant care (Table 2) [1]. For instance, in 2021, 2,318 organ transplants were performed in the Russian Federation (16.5 per million population), of which 271 were performed in children [2]. Children on waiting lists are prioritized in donor organ distribution. The problem of pediatric liver transplantation has been completely resolved, and the practice of traveling abroad for pediatric liver transplantation and for patients suffering from end-stage kidney disease has been discontinued. Operations are performed on all recipients identified and referred to transplant centers.

Low physical activity is an obvious fact that negatively affects the quality of life of both healthy people and organ recipients [3]. Low physical activity (PA) is one of the leading risk factors for the development of major non-communicable diseases such as cardiovascular diseases (CVD), type II diabetes, etc. [4]. It is these diseases that account for a high percentage of disability and deaths among the population. Studies have shown that these conditions are often side effects of immunosuppressants and glucocorticoids prescribed after transplantation, and also indicate that low PA levels are significantly associated with the risks of CVD and mortality in kidney recipients [5, 6]. According to the clinical guidelines of the European Association for the Study of the Liver (EASL): PA of liver recipients should be considered an integral part of treatment and rehabilitation [7]. In this regard, evidence-based medicine has enough facts in favor of the fact that low PA significantly worsens the quality of life of both healthy people and patients with various diseases, and especially those who have undergone such complex operations as organ transplantation [8].

Constant improvement in transplantation techniques, expansion of indications for surgical treatment of patients, including children with end-stage liver, kidney, heart, and lung diseases dictate the need to develop new and improve traditional approaches to medical rehabilitation. However, despite the urgency of the problem, rehabilitation in children after organ transplantation has practically not been developed; there are no clinical guidelines on physical activity, no criteria for dosage and prescription of physical activity in different periods after surgery have been formed.

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MEDICO-SOCIAL ASPECT OF CHILDREN'S REHABILITATION

Medico-social rehabilitation (medical, social, professional) is a process of managing patients by a complex of medical measures aimed at overcoming limitations in life.

Medical rehabilitation includes therapeutic measures aimed at restoring the patient's health. Social rehabilitation is aimed at developing the patient's skills for independence and self-care. Vocational (industrial) rehabilitation is to prepare the patient for work and going to work. But the main task is due to the necessary focus of work on types or aspects of physical activity. This includes various activities to restore the patient's ability to work. The use of therapeutic exercises, physical factors of manual therapy and reflexology.

The need for priority development of physical rehabilitation programs in children is determined by the fact that physical education and sports in childhood, i.e. before the end of a person's physical development, can significantly impact on the health of children, improving psychomotor and physical development [9].

They promote health, improve biological mechanisms of defense and adaptive reactions, and increase nonspecific resistance to various harmful environmental influences [10]. These activities should be carried out considering the peculiarities of the child's age-specific physical development, biological age, and level of physical fitness [11]. Based on the dynamics of age-related development in pediatrics, it is customary to divide children into 5 groups, where each age category has its own features in the cardiovascular, respiratory, nervous and endocrine systems. Differences between these age periods are sometimes significant because human development in childhood proceeds unevenly, heterochronically. At the same time, it is known that children who require organ transplantation initially lag behind in physical development, in the level of physical fitness, they have deviations in the health and functional state of organs and systems, their level of physical activity is reduced, and they have severe physical inactivity and hypokinesia [12]. Most children have a low level of physical activity and lead a largely sedentary lifestyle after kidney or liver transplantation, even if they are clinically stable and have no contraindications to exercise. This attitude to the motor regimen persists even many years after surgery [13].

Table 1

Number of organ transplants performed in the Russian Federation in 2013–2021

	2013	2014	2015	2016	2017	2018	2019	2020	2021
Total organs transplanted	1400	1522	1485	1704	1896	2193	2427	1960	2318
Kidney	935	1026	945	1084	1175	1361	1473	1124	1384
cadaveric	747	836	755	852	974	1161	1290	967	1183
from a living donor	188	190	190	232	201	200	183	157	201
Liver	273	302	325	378	438	505	584	559	618
cadaveric	154	176	192	229	307	341	437	390	455
from a living donor	119	126	133	149	131	164	147	169	163
Heart	164	162	179	220	252	282	335	249	290
Pancreas	16	19	12	6	6	17	10	16	10
Lungs	10	12	24	16	25	25	23	9	13
Heart-lung transplant	1	–	–	–	–	3	2	2	2
Small intestine	1	1	–	–	–	–	–	1	1

Table 2

Number of pediatric organ transplants performed in the Russian Federation in 2013–2021

	2013	2014	2015	2016	2017	2018	2019	2020	2021
Total organs transplanted	128	152	163	181	215	233	227	256	271
Kidney	57	73	65	81	105	89	101	119	122
Liver	69	76	92	96	106	133	113	131	134
Heart	2	3	6	2	3	9	11	6	15
Pancreas	–	–	–	–	–	–	–	–	–
Lungs	–	–	–	2	1	2	2	–	–
Heart-lung transplant	–	–	–	–	–	–	–	–	–
Small intestine	–	–	–	–	–	–	–	–	–

FEATURES OF PHYSICAL REHABILITATION OF CHILDREN AFTER ORGAN TRANSPLANTATION

An analysis of modern Russian and foreign literature and articles in periodicals shows that physical activity prescribed by specialist physicians and conducted by specialized personnel (physicians in therapeutic physical training, doctors in medical and physical rehabilitation, instructors and methodists in therapeutic exercises) is able to improve both the biological parameters and physical condition of an organ recipient [9, 14].

Solid organ transplantation gives many children the opportunity to lead a normal life. However, as survival rates increase, these patients are at increased risk of developing diseases associated with lifelong drug therapy [15]. For example, liver transplantation can lead to kidney disease, diabetes, infectious diseases, and childhood developmental delays. The use of high-dose corticosteroids predisposes to the development of growth and bone mineralization deficits, causing osteoporosis in children; moreover, the use of these drugs increases the risk of hypertension and diabetes in children [16]. Calcineurin inhibitors (cyclosporine or tacrolimus) can cause chronic kidney disease, infections and gastrointestinal diseases [17].

In addition, immunosuppressive therapy significantly increases the risk of skin cancer, soft tissue cancer, lymphoma, leukemia and diseases of other organs [18]. Solid organ transplantation studies have shown that these children have an increased risk of developing cardiovascular disease (hypertension, left ventricular hypertrophy and/or dilatation), obesity, dyslipidemia, and diabetes [19]. The use of immunosuppressive drugs and corticosteroids in children with kidney transplants may increase the risk of infection, osteoporosis, and secondary malignancies [20].

Kidney transplantation is expected to increase the life expectancy of children. However, many children treated with transplantation do not return to optimal health and have low physical activity. For example, after kidney transplantation, for many, the physical component of quality of life decreases while the psychological component remains unchanged, and thirty years after organ transplantation, the physical component of quality of life remains low, which affects patients' socialization [9, 15].

There are few studies evaluating the effectiveness of exercise after transplantation, but it is noted that exercise therapy is an effective method of reducing exercise-related limitations [21].

Physical activity is an effective tool for preventing cardiovascular risk factors, osteoporosis, and kidney failure [22]. It can restore both physical, functional and psychological abilities [23]. Physical activity has been demonstrated to be safe, feasible, and effective in preventing decline in quality of life in pediatric transplant recipients [24]. Regular physical activity, even light exercise, can improve exercise capacity in children with a transplanted kidney or liver and hence their quality of life [25]. Due to lack of sufficient physical activity, many patients with CKD suffer from muscle wasting, which may reduce their physical activity [26].

In a multi-year study (over 4 years), the effects of two therapeutic exercise programs in two groups among patients on hemodialysis were compared. Aerobic capacity was assessed using a modified Bruce protocol treadmill test and spiroergometric testing. One group participated in a supervised outpatient exercise training program 3 times a week on non-dialysis days, while the other group followed a training program with stationary bicycles during their dialysis sessions thrice a week. After one year and 4 years, both groups showed significant increase in aerobic endurance and considerable improvement in general well-being, which confirms the benefit and necessity of physical activity in patients with chronic kidney disease [27].

Resistance exercise can increase muscle strength, muscular endurance as well as exercise tolerance in CKD as described above [27]. For example, resistance exercise for 6 months improves skeletal muscle function and structure in patients on dialysis [26]. According to muscle biopsy data, there is an increase in the cross-sectional area of muscle fibers and new muscle fiber formation, and atrophic fibers regenerate, improving O_2 transport capacity and, consequently, exercise tolerance in patients on dialysis [26].

All this dictates the need to study the effect of physical activity on the body of children with transplanted organs, to determine the indications, contraindications to therapeutic and recreational physical training, sports, development and introduction of individual physical rehabilitation programs into clinical practice. Physical activity should become the most important part of the child's life [28].

Currently, there are no official clinical recommendations on medical rehabilitation after organ transplantation in children. Restorative measures are described only in separate guidelines on transplantation of a particular organ jointly for adults and children [29].

The clinical guidelines "*Lung transplantation, heart-lung transplantation, presence of a transplanted lung, presence of a transplanted lung, presence of a transplanted heart-lung complex, lung graft death and rejection, heart-lung graft death and rejection*", Z94.2, Z94.3, T86.3, T86.8 (adults, children), recommend strict adherence to personal hygiene, infection safety, instrumental and laboratory control, diet, as well as maintaining an optimal level of physical activity [29]. They suggest respiratory exercises for donor lung recipients

early after transplantation to improve external respiratory function with or without respiratory simulators [30], regular physical aerobic exercise to improve the function of transplanted lungs and as part of general physical health-improving measures [31]. Contraindications to exercise, according to the authors, include an unfavorable course of the postoperative period, the threat of complications from breathing exercises and/or other physical activity.

After heart transplantation, a properly designed physical and psychological rehabilitation program helps to increase adherence to drug treatment and lifestyle changes, including diet, regular physical activity and quitting smoking [21]. After determining individual exercise tolerance and assessing associated risk, carrying out a regular aerobic physical activity 3 times a week for at least 30 minutes is recommended. Patients with sedentary lifestyles should be actively encouraged to initiate low- to moderate-intensity exercise [32]. Guidelines for the use of physical rehabilitation programs in patients after heart transplantation are of great importance [33]. Recipients are recommended to undergo physical training with aerobic exercise because it improves adaptation to physical activity and contributes to modification of cardiovascular disease risk factors such as obesity, impaired glucose tolerance and hypertension [13]. However, the effect of physical training on long-term prognosis and mortality in patients after heart transplantation has not been studied.

Strength physical exercises (with weights) is a part of complex therapy for the prevention of bone mineral density loss and skeletal muscle atrophy and is recommended for patients with signs of decreased bone mineral density, as well as for the prevention of osteoporosis and undesirable effects of glucocorticoids and tacrolimus or cyclosporine drugs on muscle tissue [16].

The World Games for Children after Transplantation is a unique event in the field of medicine and sport and confirms the effectiveness of rehabilitation. These are some of the observations that most clearly demonstrate the achievements of modern transplantology. For example, Kelly Young underwent a liver transplant at the age of 7.5 months for biliary atresia. For the first time at the age of 12, Kelly competed at the World Games in 2007 (Thailand) and won 7 gold medals in swimming. Then in 2009 (Australia), she took home 5 gold medals and 1 silver medal; in 2011 (Sweden), she won 6 gold medals and 1 silver medal; and in 2017 (Spain), he cleared 4 gold and 3 bronze medals in swimming. She was recognized as an outstanding female athlete in the younger age categories from 2007 to 2015 [34].

CONCLUSION

Physical activity plays an important role in the formation of physical and psychological health in children [9, 10]. Therapeutic, health-improving physical training and sports improve the quality of life and reduce cardiovascular disease risk factors in patients with transplanted organs [13]. After organ transplantation, it is possible to lead an active lifestyle, engage in physical exercise and sports, as evidenced by various sporting events among organ recipients [35]. Improving the condition of patients after organ transplantation, increasing the life expectancy of recipients, limitations in possible labor activity, and social insufficiency determine the need [36] to combine the efforts of specialists of different profiles in the development and implementation of modern medical rehabilitation technologies in inpatient and outpatient organizations, in which the physical aspect of rehabilitation is a priority.

The medical community faces the task of providing optimal conditions for organizing and conducting medical rehabilitation activities for pediatric organ recipients in order to restore their social status and integration into society, creating conditions for the development of sports and physical training for persons with transplanted organs.

The authors declare no conflict of interest.

REFERENCES

1. *Gautier SV, Khomyakov SM.* Vedomstvennaya celevaya programma “donorstvo i transplantaciya organov v Rossijskoj Federacii”: regional’ny’j aspekt. *Russian Journal of Transplantology and Artificial Organs*. 2021; 23 (S): 6–7.
2. *Gautier SV, Khomyakov SM*. Organ donation and transplantation in the Russian Federation in 2021. 14th Report from the Registry of the Russian Transplant Society. *Russian Journal of Transplantology and Artificial Organs*. 2022; 24 (3): 8–31. [In Russ, English abstract]. https://doi.org/10.15825/1995-1191-2022-3-8-31.
3. *Masala D, Mannocci A, Unim B, Del Cimmuto A, Turchetta F, Gatto G et al*. Quality of life and physical activity in liver transplantation patients: results of a case-control study in Italy. *Transplant Proc*. 2012 Jun; 44 (5): 1346–1350. doi: 10.1016/j.transproceed.2012.01.123. PMID: 22664013.
4. *Hallal PC, Andersen LB, Bull FC, Guthold R, Haskell W, Ekelund U*. Lancet Physical Activity Series Working Group. Global physical activity levels: surveillance progress, pitfalls, and prospects. *Lancet*. 2012 Jul 21; 380 (9838): 247–257. doi: 10.1016/S0140-6736(12)60646-1. PMID: 22818937.5.
5. *Sharif A, Baboolal K.* Metabolic syndrome and solid-organ transplantation. *Am J Transplant*. 2010 Jan; 10 (1): 12–17. doi: 10.1111/j.1600-6143.2009.02882.x. Epub 2009 Dec 2. Erratum in: *Am J Transplant*. 2012 May; 12 (5): 1358. Baboolal, K [added]. PMID: 19958337.
6. *Pilmore H, Dent H, Chang S, McDonald SP, Chadban SJ*. Reduction in cardiovascular death after kidney transplantation. *Transplantation*. 2010 Apr 15; 89 (7):

851–857. doi: 10.1097/TP.0b013e3181caeead. PMID: 20048695.
7. Klinicheskie rekomendatsii EASL: transplantatsiya pecheni. *Journal of Hepatology. Russkoe izdanie*. 2016; 2 (2): 90–152; 64: 433–485. [In Russ].
8. *Oja P, Bull FC, Fogelholm M, Martin BW.* Physical activity recommendations for health: what should Europe do? *BMC Public Health*. 2010 Jan 11; 10: 10. https://doi.org/10.1186/1471-2458-10-10.
9. *Hamiwka LA, Cantell M, Crawford S, Clark CG.* Physical activity and health related quality of life in children following kidney transplantation. *Pediatr Transplant*. 2009; 13 (7): 861–867. doi: 10.1111/j.1399-3046.2009.01195.x.
10. *Biddle SJ, Asare M.* Physical activity and mental health in children and adolescents: a review of reviews. *Br J Sports Med*. 2011; 45 (11): 886–895. doi: 10.1136/bjsports-2011-090185.
11. *Larouche R, Garriguet D, Gunnell KE, Goldfield GS, Tremblay MS.* Outdoor time, physical activity, sedentary time, and health indicators at ages 7 to 14: 2012/2013 Canadian Health Measures Survey. *Health Rep*. 2016; 27 (9): 3–13.
12. *Kim JJ, Marks SD.* Long-term outcomes of children after solid organ transplantation. *Clinics (Sao Paulo)*. 2014; 69 (Suppl 1): 28–38. doi: 10.6061/clinics/2014(sup01)06. PMID: 24860856; PMCID: PMC3884158.
13. *Berben L, Engberg SJ, Rossmeissl A, Gordon EJ, Kugler C, Schmidt-Trucksäss A et al.* B-SERIOUS consortium. Correlates and Outcomes of Low Physical Activity Posttransplant: A Systematic Review and Meta-Analysis. *Transplantation*. 2019 Apr; 103 (4): 679–688. doi: 10.1097/TP.0000000000002543. PMID: 30461720.
14. *Didsbury M, McGee RG, Tong A, Craig JC, Chapman JR, Chadban S et al.* Exercise training in solid organ transplant recipients: a systematic review and meta-analysis. *Transplantation*. 2013 Mar 15; 95 (5): 679–687. doi: 10.1097/TP.0b013e31827a3d3e. PMID: 23364480.
15. *Westphal Ladfors S, Bergdahl E, Hermannsson O, Kristjansson J, Linnér T, Brandström P et al.* Longitudinal Follow-Up on Cardiopulmonary Exercise Capacity Related to Cardio-Metabolic Risk Factors in Children With Renal Transplants. *Front Sports Act Living*. 2021 Aug 16; 3: 688383. doi: 10.3389/fspor.2021.688383. PMID: 34485901; PMCID: PMC8415396.
16. *Bechtold S, Putzker S, Birnbaum J, Schwarz HP, Netz H, Dalla Pozza R.* Impaired bone geometry after heart and heart-lung transplantation in childhood. *Transplantation*. 2010 Nov 15; 90 (9): 1006–1010. doi: 10.1097/TP.0b013e3181f6300b. PMID: 20921936.
17. *Litwin M, Niemirska A.* Metabolic syndrome in children with chronic kidney disease and after renal transplantation. *Pediatr Nephrol*. 2014; 29 (2): 203–216. doi: 10.1007/s00467-013-2500-1.
18. *Lowers S.* Physical Therapy Considerations for Outpatient Treatment, Pre- and Post-Transplant. Rehabilitation for Solid Organ Transplant. 2013. *Book Physical Therapy Considerations for Outpatient Treatment, Pre- and Post-Transplant. Rehabilitation for Solid Organ Transplant*. 2013 / Editor.
19. *LaRosa C, Baluarte HJ, Meyers KE.* Outcomes in pediatric solid-organ transplantation. *Pediatr Transplant*. 2011 Mar; 15 (2): 128–141. doi: 10.1111/j.1399-3046.2010.01434.x. PMID: 21309962.
20. *Dagher M, Ng VL, Carpenter A, Rankin S, De Angelis M, Avitzur Y et al.* Overweight, central obesity, and cardiometabolic risk factors in pediatric liver transplantation. *Pediatr Transplant*. 2015 Mar; 19 (2): 175–181. doi: 10.1111/petr.12425. Epub 2015 Jan 8. PMID: 25581506.
21. *Deliva RD, Hassall A, Manlhiot C, Solomon M, McCrindle BW, Dipchand AI.* Effects of an acute, outpatient physiotherapy exercise program following pediatric heart or lung transplantation. *Pediatr Transplant*. 2012 Dec; 16 (8): 879–886. doi: 10.1111/petr.12003. Epub 2012 Oct 11. PMID: 23050737.
22. *Wolf MF, George RP, Warshaw B, Wang E, Greenbaum LA.* Physical Activity and Kidney Injury in Pediatric and Young Adult Kidney Transplant Recipients. *J Pediatr*. 2016; 179: 90–95.e2. doi: 10.1016/j.jpeds.2016.08.079.
23. *Schulz KH, Wein C, Boeck A, Rogiers X, Burdelski M.* Cognitive performance of children who have undergone liver transplantation. *Transplantation*. 2003 Apr 27; 75 (8): 1236–1240. doi: 10.1097/01.TP.0000062843.10397.32. PMID: 12717209.
24. *Thorsteinsdottir H, Diseth TH, Lie A, Tangeraas T, Matthews I, Åsberg A et al.* Small effort, high impact: Focus on physical activity improves oxygen uptake (VO_{2peak}), quality of life, and mental health after pediatric renal transplantation. *Pediatr Transplant*. 2018 Jun 19: e13242. doi: 10.1111/petr.13242. Epub ahead of print. PMID: 29921004.
25. *Krasnoff JB, Mathias R, Rosenthal P, Painter PL.* The comprehensive assessment of physical fitness in children following kidney and liver transplantation. *Transplantation*. 2006; 82 (2): 211–217. doi: 10.1097/01.tp.0000226160.40527.5f.
26. *Clapp EL, Bevington A, Smith AC.* Exercise for children with chronic kidney disease and end-stage renal disease. *Pediatr Nephrol*. 2012; 27 (2): 165–172. doi: 10.1007/s00467-010-1753-1.
27. *Kouidi E, Grekas D, Deligiannis A, Tourkantonis A.* Outcomes of long-term exercise training in dialysis patients: comparison of two training programs. *Clin Nephrol*. 2004 May; 61 Suppl 1: S31–S38. PMID: 15233245.
28. *Lui S, de Souza A, Sharma A, Fairbairn J, Schreiber R, Armstrong K et al.* Physical activity and its correlates in a pediatric solid-organ transplant population. *Pediatr Transplant*. 2020 Aug; 24 (5): e13745. doi: 10.1111/petr.13745. Epub 2020 Jun 11. PMID: 32525279.
29. Klinicheskie rekomendacii "Transplantaciya legkih, transplantaciya kompleksa "serdce–legkie", nalichie transplantirovannogo legkogo, nalichie transplantirovannogo kompleksa "serdce–legkie", otmiranie i ottorzhenie transplantata legkih, otmiranie i ottorzhenie serdechno-legochnogo transplantata" (vzroslye, deti).

Rossijskoe transplantologicheskoe obshchestvo. M., 2020; 113. [In Russ].

30. *Langer D, Burtin C, Schepers L, Ivanova A, Verleden G, Decramer M et al.* Exercise training after lung transplantation improves participation in daily activity: a randomized controlled trial. *Am J Transplant*. 2012 Jun; 12 (6): 1584–1592. doi: 10.1111/j.1600-6143.2012.04000.x. Epub 2012 Mar 5. PMID: 22390625.
31. *Walsh JR, Chambers DC, Davis RJ, Morris NR, Seale HE, Yerkovich ST et al.* Impaired exercise capacity after lung transplantation is related to delayed recovery of muscle strength. *Clin Transplant*. 2013 Jul-Aug; 27 (4): E504–E511. doi: 10.1111/ctr.12163. Epub 2013 Jul 2. PMID: 23815281.
32. *Janaudis-Ferreira T, Mathur S, Deliva R, Howes N, Patterson C, Räkel A et al.* Exercise for Solid Organ Transplant Candidates and Recipients: A Joint Position Statement of the Canadian Society of Transplantation and CAN-RESTORE. *Transplantation*. 2019 Sep; 103 (9): e220–e238. doi: 10.1097/TP.0000000000002806.
33. *Sumin A.* Physical rehabilitation of patients after heart transplantation. *Russian Heart Journal*. 2017; 16 (3): 159–167. [In Russ, English abstract]. doi: 10.18087/rhj.2017.3.2321.
34. *Shelekhova TYu, Achkasov EE, Tsirulnikova OM, Zaborova VA, Shults IM, Ahmadzai RL*. Sports for patients with transplanted organs. *Russian Journal of Transplantology and Artificial Organs*. 2018; 20 (3): 6–12. [In Russ, English abstract]. https://doi.org/10.15825/1995-1191-2018-3-6-12.
35. *Shelekhova TYu, Achkasov EE, Lazareva IA, Krumkacheva YuA, Sungatulina AA, Gautier SV*. Assessment of the quality of life of organ reoipients based on the results of the First Russian transplant games. *Russian Journal of Transplantology and Artificial Organs*. 2023; 25 (1): 62–67. [In Russ, English abstract]. https://doi.org/10.15825/1995-1191-2023-1-62-67.
36. *Vlasova-Rozanskaya EV.* Ekspertno-reabilitacionnaya pomoshch' pacientam posle transplantacii organov. *Zdravoohranenie (Minsk)*. 2016; 11: 10–17. [In Russ].

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EFFICACY OF EXTRACORPOREAL SHOCK WAVE LITHOTRIPSY FOR POST-TRANSPLANT KIDNEY STONES. HOPE OR DISAPPOINTMENT?

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Nephrolithiasis in a transplanted kidney is an important medical and social problem. The presence of renal calculi may not manifest clinically for a long time due to the peculiarities of the surgical intervention during organ transplantation. Development of chronic urinary tract infection and deterioration of the functional ability of the renal transplant in the presence of kidney stones can lead to graft death, which is an immediate threat to the patient's life. Existing Russian guidelines on the treatment of urolithiasis currently lack a clear strategy for the management of kidney transplant recipients. **Objective:** to systematize literature data on analysis of the outcomes of extracorporeal shock wave lithotripsy (ESWL) and other methods in patients with post-transplant kidney stones. **Results.** Thirty-five publications on the research topic were selected. We summarized the information on various therapy options for patients with stones in transplanted kidney: endourological approach, ESWL, percutaneous nephrolithotripsy (PCNL), open surgical treatment (nephrostomy, pyelolithotomy). A modern foreign algorithm for the management of patients with post-transplant kidney stones depending on the severity of obstruction with sepsis and the size of the renal calculi is presented. **Conclusion.** 1. The presence of stones in a kidney graft is a clinical situation that requires surgical treatment. 2. In clinical practice, different methods of treatment can be used, such as open intervention, ESWL, PCNL, retrograde transurethral manipulations. 3. In most cases, patient management tactics depend on the clinical picture (presence/absence of obstruction) and the size of the calculi. 4. The use of ESWL, as the most frequently used method, testifies to its efficiency and low-traumatic effect.

Keywords: graft, nephrolithiasis, extracorporeal shock wave lithotripsy, ureteroscopy, percutaneous nephrolithotripsy.

INTRODUCTION

Stone formation after kidney transplantation is a possible transplant complication, and can lead to chronic pyelonephritis, hydronephrosis, anuria, graft dysfunction and graft loss [1, 2]. Foreign researchers have noted that in 0.2–5.7% of cases after kidney transplantation, stones are verified in the kidney. An analysis of a retrospective study by Russian authors (1,024 cases of renal transplants) indicates the detection of renal calculi in 1.4% of the cases [3, 4]. Based on data from a large study (1994–1998) on the prevalence of nephrolithiasis in renal transplant recipients with the participation of 42,000 patients, Kevin et al. revealed that women tend to develop this complication more frequently [5].

Data from a meta-analysis by Cheungpasitporn et al. suggest that calcium salts (oxalate and phosphate) are the basis of stones in the vast majority of cases [6, 7]. The incidence of urate ranges from 0.2–10% [8].

Some researchers have suggested that renal calculi are more likely to occur within the first year after transplantation, but there has been a case reported where it took 17 years. Nephrolithiasis detected at the time of transplantation reflects cases of donor-associated nephrolithiasis and accounts for about 7% of all cases of kidney transplant nephrolithiasis [9].

Kidney graft recipients with nephrolithiasis require increased attention because of changes in kidney function and kidney innervation. A wait-and-see conservative approach to the management of patients with renal calculi <4–6 mm involves strict clinical, radiological and laboratory monitoring [7–9]. Possible therapy options include endourological approach, ESWL, PCNL and open surgical treatment (nephrostomy, pyelolithotomy)

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[10]. Existing Russian guidelines on the treatment of urolithiasis contain no clear strategy for the management of patients with transplanted kidney [12]. Therefore, the actual task is to analyze the Russian and foreign experience of various approaches to the treatment of such patients. This served as the basis for our literature review.

The **purpose** of this work is to systematize the literature on the issue of ESWL and other methods of treatment for kidney transplant stones.

MATERIALS AND METHODS

Medical literature was searched using the information and analytical databases Cochrane, Medline (part of the PubMed search engine), elibrary using text search queries "kidney transplantation", "stones in transplanted kidney", "extracorporeal shock wave lithotripsy of transplanted kidney", and "ESWL of transplanted kidney". Literature sources found were taken for further analysis according to the following criteria: date of publication, from 2000 to 2022; type of publication (in descending order of importance), meta-analyses, systematic reviews, results of randomized and non-randomized trials, registry data. A total of 35 publications meeting the inclusion criteria were selected based on the search results and were used in the analysis.

RESULTS

When analyzing the sources that were selected for writing the review, we initially considered the works that confirmed the fact that open surgical intervention in this clinical situation is considered by the researchers as an extreme measure due to the complexity and traumatic nature of the intervention [10–13]. Other approaches to the management of such patients (endourological, PCNL were less frequently considered by the authors when choosing the kidney graft stone treatment method [14–16]. The ESWL method, as the most frequently used in clinical practice, allows noninvasive intervention that does not require general anesthesia [17–19]. The use of ESWL in clinical practice demonstrates the prevalence and demand for its use, including in a special category of patients with renal transplant calculi [17–19]. Thus, in a 2006–2009 study performed at Vladimirsky Moscow Regional Research and Clinical Institute, ESWL (32 sessions) was performed in 14 patients with urolithiasis (stone size varied from 8 to 15 mm) of the transplanted kidney (the period after transplantation was from 6 months to 2 years). Evaluation of the functional state of the graft, according to the data of laboratory research methods, confirmed the feasibility of ultrasound-guided ESWL on renal calculi in this category of patients in order to prevent complications [20].

Studies by Russian scientists have confirmed the fact that ESWL is a low-traumatic procedure and comes with no side effects. Seventeen lithotripsy sessions were performed in 8 patients. Each patient had two sessions; one patient had three. Stones were detached within 1–2 weeks. No adverse reactions were detected. The efficacy of the method used was registered in all patients in 100% of cases [3].

The efficacy of ESWL, depending on stone size, location and number, was studied in the work of Spanish researchers led by Millán Rodríguez. They analyzed a cohort of patients with renal transplantation, in which 60% of the patients had multiple stones; stones were localized in the ureter (ureterovesical anastomosis) in 53% of patients, and in the bladder in 13% of patients. The authors found that the best results were achieved with a single stone, up to 13 mm in size, localized in the lower ureter [21].

In an analysis of data from 19 patients, Klingler et al. also reported the effectiveness of ESWL for caliceal stones sized 5 to 15 mm. If the stone diameter exceeded 15 mm, it was recommended to PCNL or retrograde ureterolithotripsy [22]. The same approach to the management of recipients with renal calculi in a transplanted kidney was demonstrated by Sha-dan et al. in their retrospective analysis of data from 1979–2009 [4]. A review by Challacombe et al. described 13 patients who underwent ESWL, with eight patients requiring several sessions and two patients requiring additional ureteroscopy (URS) [23].

In a study by Yuan et al., additional ESWL sessions were performed in 4 out of 5 patients, and the authors noted that due to the ectopic location of the renal graft, the pelvic bones may interfere with stone visualization and reduce the effectiveness of this treatment procedure [24].

Several published reports have recommended PCNL and antegrade endourological manipulations as the most effective methods of nephrolithiasis treatment in renal transplant recipients regardless of the stone size and its localization. For example, foreign researchers in their work summarized the results of PCNL (4760). The clinical situations considered by the authors included patients with staghorn calculi (1240), ureteric stones (85), and transplanted kidneys (14). A study of these patients with stones in transplanted kidneys demonstrated a positive effect in 89% of cases [25]. In 2013, researchers Ji et al. reported the high effectiveness of minimally invasive percutaneous laser nephrolithotripsy in 11 patients with renal transplantation [26].

There have been works that presented good outcomes of treatment of graft calculi via retrograde URS, for instance, the study by Basiri et al. [27]. Hyams et al. reported their experience with 12 patients treated exclusively by ureteroscopic intervention, seven by retrograde access and five by antegrade access, with complete stone removal achieved in 11 of 12 patients by this surgical intervention [28]. Branchereau et al. performed a retrospective review of 95 patients with graft calculi treated at 11 renal transplant centers located in different European countries. Urethroscopy was performed in 26%

of patients with a 6–24 mm stone, and no graft loss or mortality was reported with an average follow-up of 72 months [11].

In 2019, Sarier et al. reported the use of minimally invasive surgical treatment of allograft lithiasis in 22 patients, including flexible and semi-rigid URS and PCNL, without developing serious postoperative complications and with complete stone removal in 89% of cases [29].

In the work by Rebecca et al. summarized data on 2652 patients (follow-up period 2009–2020), 18 of whom underwent URS for transplanted kidney or ureteral stones; most procedures were performed using retrograde approach. In 16 of the 18 patients, a single procedure was sufficient for complete elimination of the calculus [30].

In 2012, Romain Boissier et al. performed a retrospective analysis of 37 studies involving 553 patients who underwent 20 antegrade URS, 154 retrograde URS, 118 PCNL, 25 open surgical interventions, and 155 ESWL; conservative management of patients was performed in 140 cases. The researchers noted that the stone-free rate after the procedure was 96% with open surgery, 95% with antegrade URS, 86% with PCNL, 81% with retrograde URS, and 75% with ESWL [31].

In a 2018 publication describing more than 30 years of experience in the treatment of stones after kidney transplantation based on data from 29 studies that included 42,096 patients, treatment modalities were ESWL (43.1%), active surveillance (25.4%), retrograde URS (17.6%), antegrade URS (3.9%), percutaneous nephrolithotomy (3.9%), open approach (3.9%), and urine alkalinisation (2%) [32].

A paper by X. Li et al. summarized the results of 29 studies devoted to the management of patients with stones in the renal graft; the choice of treatment tactics was determined by the clinic, localization and size of stones. The authors concluded that the use of minimally invasive procedures is optimal, and two or more such procedures can be used to increase the effectiveness of treatment and accelerate recovery processes in the postoperative period [10].

DISCUSSION

There are a number of causes of formation of stones in a graft, such as thyroid dysfunction (imbalance of hormones T3, T4), ureteral obstruction accompanied by urinary stasis, presence of a foreign body (for example, non-absorbable suture material), metabolic disorders (gout, hyperuricemia, etc.) [12]. Continuous intake of medications such as immunosuppressants increases serum and urine uric acid levels, which may also lead to stone formation. Some researchers have reported an association between stone formation after kidney transplan-

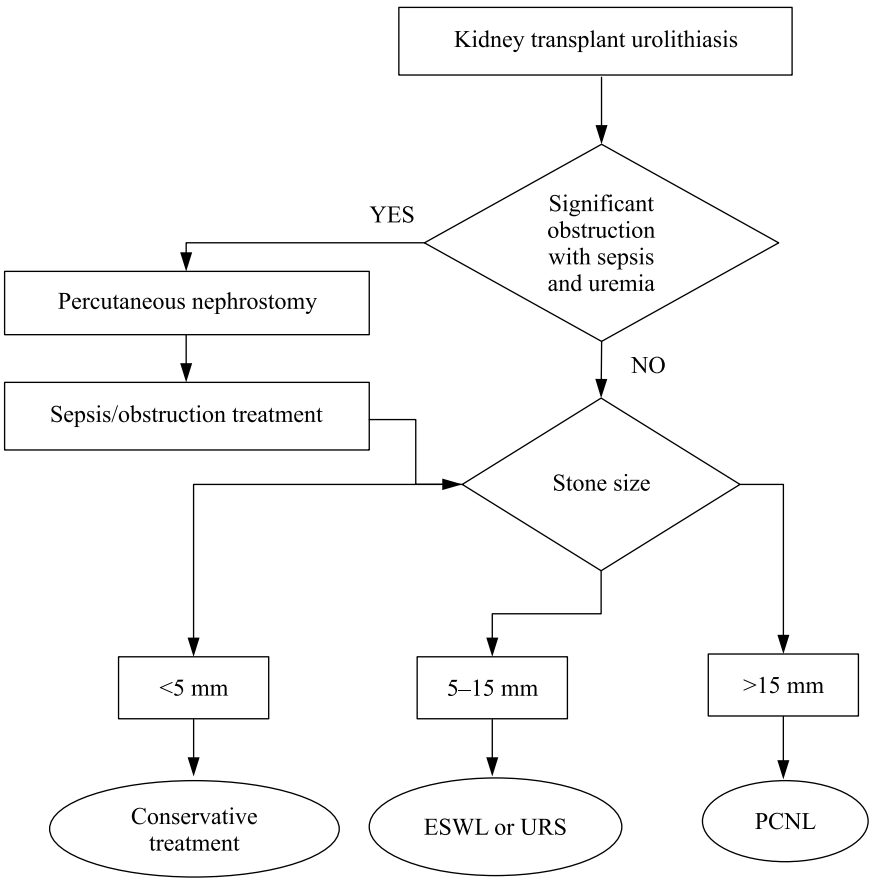

Fig. Flowchart of management of kidney transplant recipients with kidney stones (Adapted from Mohammadi, 2021 [36])

tation and age, gender, and tobacco smoking in donors and recipients [33, 34].

Changes in the treatment tactics for patients with renal calculi is worth noting. Initially, when the pathological process was asymptomatic, preference was given to conservative management, including dynamic monitoring and ultrasound examination of the kidneys. Later, removal of stones from the transplanted kidney even in the absence of clinical symptoms in the recipient was considered optimal. Proponents of this approach explain its prospects by the steady deterioration of the function of the transplanted kidney and the development of chronic urinary tract infection in the presence of renal calculi, which could eventually lead to graft death [35].

Determining the approach to surgical treatment tactics is a complex task, since many factors need to be taken into account: the polyetiology of the pathology, concomitant changes in the graft tissue, ongoing therapy, etc.

Initially, open techniques (nephrostomy, pyelolithotomy) were popular. However, it should be emphasized that open surgical intervention has a number of drawbacks: preparation of the kidney and ureter is a difficult task due to their topographical features; postoperative complications (infection, fistula formation, pain syndrome, etc.) are possible [11, 13]. In addition, open surgical intervention can be associated with pronounced cicatricial adhesion around the transplanted kidney.

The use of ESWL (as both monotherapy and combined treatment) in clinical practice is justified and effective for kidney and ureteral stones ≤1.5 cm in size. For calculi >1.5 cm, this technique is usually combined with renal catheterization, placement of an internal stent or (less frequently) percutaneous puncture nephrostomy (Fig.) [12].

CONCLUSIONS

1. The presence of kidney stones in kidney transplant recipients is a clinical situation that requires surgical treatment.
2. In clinical practice, different treatment methods can be used: open intervention, ESWL, PCNL, retrograde transurethral manipulations.
3. In most cases, patient management tactics are determined by the clinical picture (presence/absence of obstruction) and size of the calculi.
4. The use of ESWL, as the most common method, testifies to its effectiveness and low traumaticity.

The authors declare no conflict of interest.

REFERENCES

1. *Palazzo S, Colamonico O, Forte S, Matera M, Lucarelli G, Ditonno P et al*. Experience of percutaneous access under ultrasound guidance in renal transplant patients with allograft lithiasis. *Arch Ital Urol Androl.* 2016; 88 (4): 337–340. doi: 10.4081/aiua.2016.4.337.
2. *Markić D, Krpina K, Ahel J, Gršković A, Španjol J, Rubinić N et al.* Treatment of Kidney Stone in a Kidney-Transplanted Patient with Mini-Percutaneous Laser Lithotripsy: A Case Report. *Case Rep Nephrol Dial*. 2016; 6 (1): 26–31. doi: 10.1159/000444251.
3. *Lopatkin NA, Trapeznikova MF, Perlin DV.* Urologicheskie oslozhneniya pri transplantatsii pochki. M., 2004: 174–190.
4. *Li SD, Wang QT, Chen WG.* Treatment of urinary lithiasis following kidney transplantation with extracorporeal shock-wave lithotripsy. *Chinese Medical Journal*. 2011; 124 (9): 1431–1434.
5. *Abbott KC, Schenkman N, Swanson SJ, Agodoa LY.* Hospitalized nephrolithiasis after renal transplantation in the United States. *Am J Transplant*. 2003; 3 (4): 465–470. doi: 10.1034/j.1600-6143.2003.00080.x.
6. *Cheungpasitporn W, Thongprayoon C, Mao MA, Kittanamongkolchai W, Jaffer Sathick IJ, Dhondup T, Erickson SB.* Incidence of kidney stones in kidney transplant recipients: a systematic review and meta-analysis. *World J Transplant*. 2016; 6 (4): 790–797. doi: 10.5500/wjt.v6.i4.790.
7. *Santillán D, Scherñuk Schroh JC, Gutierrez PA, Thomas F, Tirapegui FI, Moldes J et al*. Case Report: Mini-endoscopic combined intrarenal surgery in an en-bloc kidney transplant. *Afr J Urol*. 2021; 27 (1): 147. doi: 10.1186/s12301-021-00249-4.
8. *Kim H, Cheigh JS, Ham HW*. Urinary stones following renal transplantation. *Korean J Intern Med*. 2001; 16 (2): 118–122. doi: 10.3904/kjim.2001.16.2.118.
9. *Bolen E, Stern K, Humphreys M, Brady A, Leavitt T, Zhang N, Keddis M.* Urine metabolic risk factors and outcomes of patients with kidney transplant nephrolithiasis. *Clinical Kidney Journal*. 2021; 15 (3): 500–506. doi: 10.1093/ckj/sfab208.
10. *Li X, Li B, Meng Y, Yang L, Wu G, Jing H et al.* Treatment of recurrent renal transplant lithiasis: analysis of our experience and review of the relevant literature. *BMC Nephrol*. 2020; 21 (1): 238. doi: 10.1186/s12882-020-01896-5.
11. *Branchereau J, Timsit MO, Neuzillet Y, Bessède T, Thuret R, Gigante M et al*. Management of renal transplant urolithiasis: A multicentre study by the French Urology Association Transplantation Committee. *World J Urol*. 2018; 36 (1): 105–109. doi: 10.1007/s00345-017-2103-8.
12. *Mohammadi A, Nikoobakht MR, Hosseini SR*. Urolithiasis in Renal Transplantation Patients: An Update of the Literature. *Translational Research in Urology*. 2021; 3 (4): 149–153. doi: 10.22034/TRU.2021.305308.1081.
13. *Geraghty R, Wood K, Sayer JA*. Calcium oxalate crystal deposition in the kidney: identification, causes and consequences. *Urolithiasis.* 2020; 48 (5): 377–384. doi: 10.1007/s00240-020-01202-w.
14. *Eslahi A, Salehipour M, Hosseini MM, Ahmed F, Hosseini SH, Ghafari S.* Mini-percutaneous nephrolithotomy via ultrasound guidance in transplanted kidney: a report of two cases. *Pan Afr Med J*. 2022; 41: 333. doi: 10.11604/pamj.2022.41.333.33169.

15. *He Z, Li X, Chen L, Zeng G, Yuan J.* Minimally invasive percutaneous nephrolithotomy for upper urinary tract calculi in transplanted kidneys. *BJU Int.* 2007; 99 (6): 1467–1471. doi: 10.1111/j.1464-410X.2007.06768.x.
16. *Kadlec AO, Ross MJ, Milner JE.* Mini-percutaneous nephrolithotomy with ureteral access sheath in a transplanted kidney: case report and literature review. *Urol Int.* 2013; 91 (2): 236–238. doi: 10.1159/000343987.
17. *Garg M, Johnson H, Lee SM, Rai BP, Somani B, Philip J.* Role of Hounsfield Unit in Predicting Outcomes of Shock Wave Lithotripsy for Renal Calculi: Outcomes of a Systematic Review. *Current Urology Reports*. 2023; 24 (4): 173–185. doi: 10.1007/s11934-023-01145-w.
18. *Serrell EC, Best SL.* Imaging in stone diagnosis and surgical planning. *Current Opinion in Urology.* 2022; 32 (4): 397–404. doi: 10.1097/MOU.0000000000001002.
19. *Elbaset MA, Taha DE, Anas M, Abouelkheir RT, Edwan M, Abdullateef M et al*. Optimization of shockwave lithotripsy use for single medium sized hard renal stone with stone density ≥1000 HU. A prospective study. *World J Urol*. 2022; 40 (1): 243–250. doi: 10.1007/s00345-021-03807-1.
20. *Lubennikov AE, Trushkin RN, Artyukhina LYu*. Nefrolitiaz u retsipientov pochechnogo transplantata. *Klinicheskaya nefrologiya*. 2016; (3–4): 69–74.
21. *Millán Rodríguez F, González de Chaves E, Rousaud Barón F, Izquierdo Latorre F, Rousaud Barón A.* Treatment of urinary calculi in transplanted kidney with extracorporeal shock wave lithotripsy. *Arch Esp Urol*. 2003; 56 (7): 793–798.
22. *Klingler HC, Kramer G, Lodde M, Marberger M.* Urolithiasis in allograft kidneys. *Urology*. 2002; 59 (3): 344–348. doi: 10.1016/s0090-4295(01)01575-8.
23. *Challacombe B, Dasgupta P, Tiptaft R, Glass J, Koffman G, Goldsmith D, Khan MS.* Multimodal management of urolithiasis in renal transplantation. *BJU Int.* 2005; 96 (3): 385–389. doi: 10.1111/j.1464-410X.2005.05636.x.
24. *Yuan HJ, Yang DD, Cui YS, Men CP, Gao ZL, Shi L, Wu JT.* Minimally invasive treatment of renal transplant nephrolithiasis. *World J Urol*. 2015; 33 (12): 2079–2085. doi: 10.1007/s00345-015-1549-9.
25. *Li X, He Z, Wu K, Li SK, Zeng G, Yuan J et al*. Chinese minimally invasive percutaneous nephrolithotomy: the Guangzhou experience. *J Endourol*. 2009; 23 (10): 1693–1697. doi: 10.1089/end.2009.1537.
26. *Ji ZG, Tian Y, Chen YQ, Liu Z, Lin J, Wang JM et al*. A Retrospective Study of minipercutaneous laser lithotripsy for treatment of allograft kidney Lithiasis Obstruction. *Transpl Proc*. 2013; 45 (9): 3298–3301. doi: 10.1016/j.transproceed.2013.06.007.
27. *Basiri A, Nikoobakht MR, Simforoosh N, Hosseini Moghaddam SM.* Ureteroscopic management of urological complications after renal transplantation. *Scand J Urol Nephrol*. 2006; 40 (1): 53–56. doi: 10.1080/00365590510007838.
28. *Hyams E, Marien T, Bruhn A, Quirouet A, Andonian S, Shah O, Matlaga B.* Ureteroscopy for transplant lithiasis. *J Endourol*. 2012; 26 (7): 819–822. doi: 10.1089/end.2011.0495.
29. *Sarier M, Duman I, Yuksel Y, Tekin S, Demir M, Arslan F et al*. Results of minimally invasive surgical treatment of allograft lithiasis in liver donor renal transplant recipients: A single center experience of 3758 renal transplantations. *Urolithiasis*. 2019; 47 (3): 273–278. doi: 10.1007/s00240-018-1051-0.
30. *Gerber RC, Best SL, Hedican SP, Nakada SY.* Flexible Ureteroscopy as the New Standard for the Management of Renal Transplant Urolithiasis <15 mm: A Single-Center Experience. *Journal of Endourology*. 2021 Oct; 35 (10): 1443–1447. doi: 10.1089/end.2020.0473.
31. *Boissier R, Rodriguez-Faba O, Zakri RH, Hevia V, Budde K, Figueiredo A et al.* Evaluation of the Effectiveness of Interventions on Nephrolithiasis in Transplanted Kidney. *Eur Urol Focus*. 2023 May; 9(3): 491–499. doi: 10.1016/j.euf.2022.11.019.
32. *Emiliani E, Subiela JD, Regis F, Angerri O, Palou J.* Over 30-yr experience on the management of graft stones after renal transplantation. *Eur Urol Focus*. 2018; 4 (2): 169–174. doi: 10.1016/j.euf.2018.06.007.
33. *Tamadon MR, Nassaji M, Ghorbani R.* Cigarette smoking and nephrolitiasis in adult individuals. *Nephrourol Mon*. 2013; 5 (1): 702–705. doi: 10.5812/numonthly.5251.
34. *Chen CH, Lee JI, Jhan JH, Lee YC, Geng JH, Chen SC et al*. Secondhand smoke increases the risk of developing kidney stone disease. *Sci Rep*. 2021; 11 (1): 17694. doi: 10.1038/s41598-021-97254-y.
35. *Assimos D, Krambeck A, Miller NL, Monga M, Murad MH, Nelson CP et al*. Surgical Management of Stones: American Urological Association/Endourological Society Guideline, Part I. *J Urol*. 2016; 196: 1153–1160. doi: 10.1016/j.juro.2016.05.090.

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CLINICAL CASE OF LONG-TERM MECHANICAL CIRCULATORY SUPPORT IN A PATIENT WITH BIVENTRICULAR HEART FAILURE AFTER CARDIAC STAB WOUND

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Cardiac injury remains one of the most complex conditions in emergency surgery. Only 6% of patients with penetrating heart wounds manage to be delivered to the operating room for surgery, and the mortality rate is still extremely high. Unfortunately, such emergency interventions are often performed in institutions lacking the ability to provide the full range of reconstructive techniques, resulting in suboptimal correction and a high risk of developing postoperative complications. This paper describes a clinical case of successful repair of multiple stab wounds to the heart with concomitant anterior descending artery injury followed by severe heart failure requiring biventricular mechanical circulatory support.

Keywords: cardiac injury, mechanical circulatory assist device, heart failure, assisted circulation, biventricular support.

INTRODUCTION

Despite the fact that heart injury was first mentioned in medical literature 3,000 years BC, this condition is still one of the most complex in emergency surgery practice [1]. An analysis of 1,198 cases of penetrating heart wounds showed that only 6% of patients with such wounds manage to get to the operating room for surgical treatment, with a mortality rate of 70–90% [2–4]. The location of two-thirds of the heart mass to the left of the midline forms a statistically decreasing frequency of damage to the chambers of the right ventricle (40–43%), left ventricle (34–40%), right atrium (18–24%) and left atrium (3–5%) [3].

Coronary artery injury is not the most common companion of penetrating heart wounds, occurring only in 3–9% of cases. However, these figures indicate only the occurrence in those patients who were on the operating table and underwent surgical treatment [5, 6]. A combination of penetrating wounds of the cardiac cavities with damage to the major coronary artery branches is an extremely difficult condition to correct in emergency surgery. Patients with such wounds require prompt diagnosis and treatment in the nearest hospital. Unfortunately, such emergency interventions are often performed in institutions that do not have the capacity to provide a full range of reconstructive techniques. Therefore, elimination of heart wall defects and bleeding becomes the main goal of emergency care. Stopping bleeding in coronary artery injury in most cases is limited to simple ligation or stitching, which leads to myocardial infarction in 90% of cases [7].

This report describes a clinical case of successful repair of multiple stab wounds of the heart with concomitant damage to the anterior descending artery, followed by severe heart failure, which required biventricular mechanical circulatory support.

CLINICAL CASE

A 21-year-old female patient (weight 53 kg, height 179 cm) was criminally assaulted with six penetrating stab wounds to the chest. The patient was rushed to the nearest surgical hospital. After performing left anterolateral thoracotomy and chest revision, three penetrating stab wounds in the left ventricular cavity, with damage to the anterior descending artery in the middle third, were found.

During surgical intervention, due to the urgency of the condition and massive bleeding, the anterior descending artery was sutured, resulting in an early postoperative period that was complicated by acute myocardial infarction. After discharge from the hospital 11 months later, the patient was hospitalized again with signs of progressive heart failure, dyspnea, pulmonary edema and ascites. Echocardiography revealed dilatation of all heart chambers, akinesis of segments 2, 4, 8, 9, 10, 11, 13, 14, 17, reduced left ventricular (LV) and right ventricular (RV) contractile function; LV ejection fraction (EF) was 18%, LV end-diastolic volume (EDV) was 220 mL, RV EF was 18–20%, grade 2–3 mitral regurgitation, and

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flow area was 8.1 cm^2; there was pulmonary hypertension (PH) with mean pulmonary arterial pressure being 41 mmHg and pulmonary vascular resistance (PVR) 9.9 Wood's units.

Considering biventricular heart failure, PH and high risk of posttransplant contractile dysfunction of the transplanted heart, the patient underwent concurrent implantation of two mechanical circulatory support devices (Sputnik, Russia) in a biventricular configuration. Left ventricular assist device (LVAD) was implanted using a standard technique according to the "left ventricular apex-to-aorta" model. The right ventricular assist device (RVAD) was connected according to the "right atrium-to-pulmonary trunk" model. A two-stage venous cannula (Medtronic Inc., 34/46 French), inserted into the superior vena cava through the right atrial wall, was used as an inflow cannula. The RVAD outflow line was anastomosed to the pulmonary artery trunk as shown in Figure.

At day 34 of hospital stay, with mechanical circulatory assist devices functioning in a stable condition, PVR decreased to 1.9 Wood units, the patient was placed on the heart transplant waitlist and discharged home. Five months after implantation of the biventricular assist device (BiVAD), multiple alarms were triggered on the LVAD controller. Against the alarms, there was a significant deterioration in well-being and the appearance of heaviness in the heart area. The patient was hospitalized with suspected mechanical circulatory support dysfunction. On examination, RVAD cavity thrombosis was detected. At the same time, the patient's condition remained stable during the LVAD functioning. Doppler echocardiography showed LV contractile function to be normal; LV EF, 62%; LV EDV, 98 mL. RV myocardial contractility was not decreased, RV EF was 44%, RV EDV was 42 mL. Estimated systolic pulmonary artery pressure was 39 mmHg, mean pulmonary arterial pressure was 32 mmHg. Grade 1–2 mitral regurgitation, flow area was 5.4 cm^2, S flow / S of left atrium was 20.53%. After discharge from the hospital, the patient was active and had no complaints. However, due to food poisoning, against the background of hypocoagulation, an extensive hemorrhagic stroke developed. This led to the death of the patient 9 months after the mechanical circulatory support device was implanted.

DISCUSSION

The incidence of penetrating heart wounds has increased over the past few years, with stab and gunshot wounds predominating. According to A. Isaza-Restrepo et al., the mortality rate in gunshot and stab wounds to the heart is 54.5% and 18% respectively (p = 0.0120) [8]. Despite the high mortality rate in cardiac wounds, even successful management of this life-threatening condition is associated with a high risk of complications. For example, in a clinical case, where multiple penetrating heart wounds, described by us, were sutured successfully, coronary bed and valve damage resulted in biventricular heart failure that required orthotopic heart transplantation (OHT). However, OHT was contraindicated due to high PVR, which, according to literature, is associated with a high risk of graft dysfunction and early death [9, 10].

LVAD implantation can reverse PH within certain limits in OHT candidates as part of a bridge-to-transplantation strategy [11–14]. Thanks to the development of assisted circulation technology, OHT has become available to recipients previously considered unsuitable for this operation [15–17]. The possibility of preparing a recipient with PH for OHT using circulatory support techniques has also been shown in the clinical case we have described. The use of prolonged biventricular mechanical circulatory support contributed to a decrease in blood pressure and pulmonary circulation resistance one month after implantation. This allowed to list the patient for heart transplantation. It is also curious that RV myocardial contractility was restored against the background of thrombosis and RVAD withdrawal with compensation of hemodynamic parameters.

Optimal timing of OHT, especially in patients with PH and an implanted LVAD, remains a subject of research. According to Mikus et al., PVR reduces in the first 6 months after LVAD implantation, and longer support does not add useful effects on hemodynamic parameters of pulmonary circulation [11]. At the same time, according to Moayedifar et al., long-term posttransplant survival in patients with fixed PH who were successfully bridged to candidacy for heart transplantation with LVAD implantation was 83.5% and 81.0% at 3 and 5 years, respectively. This was comparable with the survival rate in patients who underwent OHT on the

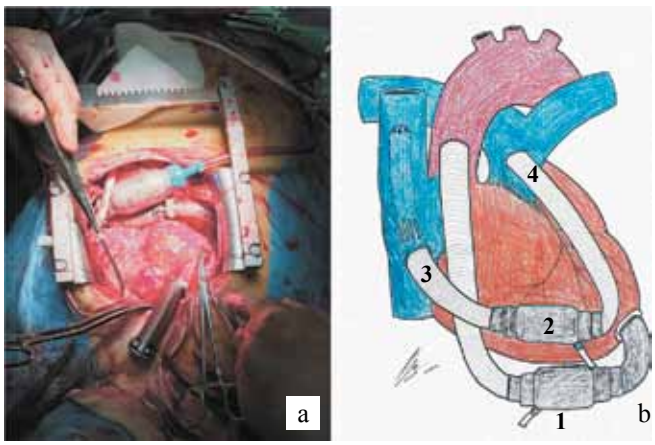

Fig. Concurrent implantation of two mechanical circulatory assist devices in a biventricular configuration. a, view of the operating wound; b, implantation scheme; 1, LVAD; 2, RVAD; 3, RVAD inflow cannula; 4, RVAD outflow cannula

background of earlier implanted LVAD for reasons not related to PH (3 years: 87.5%, 5 years: 85.4%) [18]. These survival rates were comparable to OHT outcomes in PH-free patients, 84% and 75% at year 1 and 5, respectively [19].

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REFERENCES

1. *Asensio JA, Soto SN, Forno W, Roldan G, Petrone P, Salim A et al.* Penetrating cardiac injuries: a complex challenge. *Injury*. 2001; 32 (7): 533–543. doi: 10.1016/s0020-1383(01)00068-7.
2. *Carr JA, Buterakos R, Bowling WM, Janson L, Kralovich KA, Copeland C et al.* Long-term functional and echocardiographic assessment after penetrating cardiac injury: 5-year follow-up results. *J Trauma*. 2011; 70 (3): 701–704. doi: 10.1097/TA.0b013e31820c405a.
3. *Kang N, Hsee L, Rizoli S, Alison P.* Penetrating cardiac injury: overcoming the limits set by Nature. *Injury*. 2009; 40 (9): 919–927. doi: 10.1016/j.injury.2008.12.008.
4. *Campbell NC, Thomson SR, Muckart DJ, Meumann CM, Van Middelkoop I, Botha JB.* Review of 1198 cases of penetrating cardiac trauma. *Br J Surg*. 1997; 84 (12): 1737–1740.
5. *Tector AJ, Reuben CF, Hoffman JF, Gelfand ET, Keelan M, Worman L.* Coronary artery wounds treated with saphenous vein bypass grafts. *JAMA*. 1973; 225 (3): 282–284.
6. *Lobay KW, MacGougan CK.* Traumatic coronary artery dissection: a case report and literature review. *J Emerg Med*. 2012; 43 (4): e239–e243. doi: 10.1016/j.jemermed.2010.04.019.
7. *Reissman P, Rivkind A, Jurim O, Simon D.* Case report: the management of penetrating cardiac trauma with major coronary artery injury – is cardiopulmonary bypass essential? *J Trauma*. 1992; 33 (5): 773–775. doi: 10.1097/00005373-199211000-00031.
8. *Isaza-Restrepo A, Bolívar-Sáenz DJ, Tarazona-Lara M, Tovar JR.* Penetrating cardiac trauma: analysis of 240 cases from a hospital in Bogota, Colombia. *World J Emerg Surg*. 2017; 12: 26. doi: 10.1186/s13017-017-0138-1.
9. *Kirklin JK, Naftel DC, Kirklin JW, Blackstone EH, White-Williams C, Bourge RC.* Pulmonary vascular resistance and the risk of heart transplantation. *J Heart Transplant*. 1988; 7 (5): 331–336.
10. *Erickson KW, Costanzo-Nordin MR, O'Sullivan EJ, Johnson MR, Zucker MJ, Pifarré R et al.* Influence of preoperative transpulmonary gradient on late mortality after orthotopic heart transplantation. *J Heart Transplant*. 1990; 9 (5): 526–537.
11. *Mikus E, Stepanenko A, Krabatsch T, Loforte A, Dandel M, Lehmkuhl HB et al.* Reversibility of fixed pulmonary hypertension in left ventricular assist device support recipients. *Eur J Cardiothorac Surg*. 2011; 40 (4): 971–977. doi: 10.1016/j.ejcts.2011.01.019.
12. *Mehra MR, Canter CE, Hannan MM, Semigran MJ, Uber PA, Baran DA et al.* The 2016 International Society for Heart Lung Transplantation listing criteria for heart transplantation: A 10-year update. *J Heart Lung Transplant*. 2016; 35 (1): 1–23. doi: 10.1016/j.healun.2015.10.023.
13. *Khvan DS, Chernyavsky AM, Efendiev VU, Sirota DA, Doronin DV, Fomichev AV et al.* A case of the "Berlin Heart EXCOR" biventricular assist device implantation as a bridge to heart transplantation for a patient with dilated cardiomyopathy and Terminal Heart Failure. *Russian Journal of Transplantology and Artificial Organs*. 2018; 20 (2): 69–73.
14. *Fomichev AV, Khvan DS, Agaeva HA, Zhulkov MO, Doronin DV, Chernyavsky AM.* Experience of heart transplantation with an extended cold ischemic time of Donor Heart. *Russian Journal of Cardiology*. 2020; 25 (8): 4011.
15. *Adamson RM, Dembitsky WP, Jaski BE, Daily PO, Moreno R, Kim JC et al.* Left ventricular assist device support of medically unresponsive pulmonary hypertension and aortic insufficiency. *ASAIO J*. 1997; 43 (4): 365–369.
16. *Haddad H, Elabbassi W, Moustafa S, Davies R, Mesana T, Hendry P et al.* Left ventricular assist devices as bridge to heart transplantation in congestive heart failure with pulmonary hypertension. *ASAIO J*. 2005; 51 (4): 456–460. doi: 10.1097/01.mat.0000169125.21268.d7.
17. *Martin J, Siegenthaler MP, Friesewinkel O, Fader T, van de Loo A, Trummer G et al.* Implantable left ventricular assist device for treatment of pulmonary hypertension in candidates for orthotopic heart transplantation – a preliminary study. *Eur J Cardiothorac Surg*. 2004; 25 (6): 971–977. doi: 10.1016/j.ejcts.2004.01.052.
18. *Moayedifar R, Zuckermann A, Aliabadi-Zuckermann A, Riebandt J, Angleitner P, Dimitrov K et al.* Long-term heart transplant outcomes after lowering fixed pulmonary hypertension using left ventricular assist devices. *Eur J Cardiothorac Surg*. 2018 1; 54 (6): 1116–1121. doi: 10.1093/ejcts/ezy214.
19. *Lund LH, Edwards LB, Kucheryavaya AY, Benden C, Christie JD, Dipchand AI et al.* The registry of the International Society for Heart and Lung Transplantation: thirty-first official adult heart transplant report – 2014; focus theme: retransplantation. *J Heart Lung Transplant*. 2014; 33 (10): 996–1008. doi: 10.1016/j.healun.2014.08.003.

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EXPERIENCE OF OUTPATIENT FOLLOW-UP OF HEART TRANSPLANT RECIPIENTS AT SHUMAKOV CENTER

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Heart transplantation (HT) is considered the optimal therapy for end-stage heart failure. In recent years, the number of operations performed has been growing, which has led to a rise in the number of heart transplant recipients requiring outpatient follow-up. **Objective:** to evaluate the effectiveness of the model of dual personalized follow-up of heart transplant recipients in the consultative and diagnostic department of Shumakov National Medical Research Center of Transplantology and Artificial Organs. **Materials and methods.** The study included 1,436 patients under outpatient follow-up from January 2008 to December 2022. Recipient data, results of laboratory and instrumental examination methods, nature and frequency of complications at different follow-up periods were analyzed. **Results:** At the time of discharge from the hospital, 98.7% of patients had received triple-drug immunosuppressive therapy; 6 months later, methylprednisolone was discontinued in 72.2% of recipients. Mean tacrolimus level during the 1-year follow-up was 8.7 ± 2.7 ng/mL; in the period from 1 to 5 years of follow-up, the mean was 5.1 ± 2.4 ng/mL. At year 1 after transplantation, 23 (1.7%) recipients had been converted to everolimus; by the end of year 5 of follow-up, the number had increased to 8.6%. The most frequently detected complications during outpatient follow-up were: hypertension (48.65%), post-transplant diabetes mellitus (7.24%), nephropathy (35.97%), and malignant neoplasms (4.2%). Recipient survival, excluding in-hospital mortality, was 96.5%; and 88.0% at year 1 and 5 of follow-up, respectively. **Conclusion:** The dual personalized approach model for outpatient follow-up and treatment of heart transplant recipients will improve recipient survival and quality of life in the long-term post-HT period.

Keywords: heart transplantation, vasculopathy, kidney failure, post-transplant diabetes mellitus, malignant neoplasms, hypertension.

INTRODUCTION

Heart transplantation (HT) is a high-tech medical care for patients with end-stage chronic heart failure. It is aimed at prolonging and improving the quality of life in this patient cohort [1]. In Russia, the first successful HT was performed on March 12, 1987 at Shumakov National Medical Research Center of Transplantology and Artificial Organs ("Shumakov Center"). Since that time, the research center has taken a leading role in providing transplantation care to patients with end-stage organ diseases, including the heart [2, 3]. Apart from surgical care, the ideology of the research center involves monitoring organ recipients in the long-term postoperative period, which allows to keep statistical records and analyze the survival rate in the post-transplant long-term period. Today, against the background of improving the organization of organ donation in our country, improving peri- and postoperative management of heart recipients, and the emergence of new effective immunosuppressive agents, the number of orthotopic heart transplant operations performed has doubled. This has led to a threefold increase in the number of recipients living with a transplanted heart. By early 2018, the number of such recipients had exceeded 800 [4]. Follow-up of heart transplant recipients after discharge from the hospital involves a multidisciplinary approach to improve their quality of life, prevent complications and ensure early detection of complications that emerge at different periods after surgical intervention. The outcomes of heart transplantation in the long-term follow-up period depend, among other things, on the professional management of the recipient at the outpatient follow-up stage. The role of the outpatient physician includes deciding on the frequency of visits, monitoring immunosuppressive therapy, determining indications for hospitalization, explaining certain treatment guidelines, encouraging adherence to treatment and lifestyle modification.

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In our country, Shumakov Center is the leading institution in providing this type of medical care [2]. Therefore, the center has unique experience in outpatient follow-up of heart transplant recipients.

The aim of our work is to evaluate the effectiveness of the model of dual personalized follow-up of heart transplant recipients at the Consultative and Diagnostic Department, Shumakov Center.

MATERIALS AND METHODS

After discharge from the hospital, the patients' health status was monitored by a cardiologist at the Consultative and Diagnostic Department, Shumakov Center, as well as by health care specialists at the patient's place of residence. Physicians from Shumakov Center improve their professional level of training, undergoing regular training, on-the-job training, and also participate in Russian and foreign conferences and congresses. The procedure for follow-up of heart transplant recipients was developed empirically, based on long-term experience monitoring this patient cohort (see Table 1). Specialists from Shumakov Center conducted remote consultations with local physicians and/or heart transplant recipients by telephone or via the Internet. Annually and if there were indications for hospitalization, the recipients were hospitalized in the cardiology ward of Shumakov Center for more in-depth examination and correction of drug therapy.

All patients underwent routine general clinical examination: history taking, physical examination methods, as well as the necessary range of instrumental and laboratory methods of diagnosing graft function: echocardiography to assess graft function, electrocardiography to detect rhythm problems. Where necessary, additional examination methods were performed depending on the patient's current condition.

All patients received multicomponent immunosuppressive therapy including a combination of calcineurin inhibitors (tacrolimus, Tac), cytostatics (mycophenolic acid or mycophenolate mofetil (MMF)) or proliferative signaling inhibitors (everolimus, EV), methylprednisolone. Drug dosage depended on the time since the surgical intervention and graft rejection frequency. Delayed conversion to EV was performed in case of chronic graft rejection (cardiac graft vasculopathy), progression of renal failure on the background of long-term intake of calcineurin inhibitors (CNI) and detection of malignant neoplasms. The side effects of immunosuppressive therapy were evaluated based on glomerular filtration rate (GFR) level; assessment of neurological status; oncological screening and others.

Immunosuppressive therapy was monitored by assessing the target serum levels of immunosuppressive drugs on a Cobas e411 analyzer (Roche, Switzerland) by electrochemiluminescence immunoassay.

Coronary angiography and endomyocardial biopsies were performed during the first week after surgical intervention, then according to the examination schedule or as indicated. Acute cellular rejection was diagnosed based on the results of histochemical study of endomyocardial biopsies. The diagnosis of antibody-mediated rejection was made according to the ISHLT-2013 classification (M.E. Hammond et al., 2016).

Data are presented as arithmetic mean and standard deviation (M ± SD). The Kaplan–Meier survival regression analysis method (IBM SPSS Statistics 23) was used to estimate event-free survival.

RESULTS

For the period from January 2008 to December 2022, 1,775 heart transplants were performed at Shumakov Center. This included 51 heart retransplants and 4 heart re-retransplants. Cases of heart retransplantation, hospital mortality, and recipients under 18 years of age were excluded from the study. Thus, the study included 1,436 heart transplant recipients who underwent outpatient follow-up at Shumakov Center from January 2008 to December 2022.

One of the tasks of outpatient follow-up is to assess the efficacy and safety of ongoing immunosuppressive therapy. The efficacy of therapy was evaluated based on the results of endomyocardial biopsies performed. Safety was assessed based on the obtained blood levels of CNI and proliferative signaling inhibitors, as well as detection of side effects on the background of long-term use of immunosuppressive drugs. At the time of hospital

Table 1

Outpatient follow-up plan and planned admission of heart transplant recipients

	Post-HT timeline				
	First 4 weeks	1–3 months	4 months – 1 year	1–5 years	>5 years
Outpatient visit	Once a week	Once every 2 weeks	Once every 3 months	Once every 3–6 months	Every 6 months
Blood test	Once a week	Once every 2 weeks	Once every 3 months	Once every 3 months	Once every 6 months
Heart biopsy	–	–	Once every 6 months	Once a year	Once a year
ECHOCG	Once a week	Once every 3 months	Once every 3 months	Once every 6 months	Once every 6 months
Coronary angiography	–	Once a year	Once a year	Once a year	Once a year

discharge, 98.7% of patients (n = 1,418) received triple-drug immunosuppressive therapy – Tac, MMF, and methylprednisolone; 1.3% (n = 18) of recipients received double-drug immunosuppressive therapy (Tac + MMF).

In case of planned postoperative period, low immunologic risk and absence of data on acute graft rejection crises. Immunosuppressive therapy was adjusted 6 months after operation. So, methylprednisolone was withdrawn in 1,123 (72.2%) recipients, the rest 313 (21.8%) continued to receive triple-drug immunosuppressive therapy.

Analysis of Tac serum levels showed that mean serum level of the drug at year 1 of follow-up was 8.7 ± 2.7 ng/mL. Subsequently, the drug dose was reduced, which resulted in lower Tac levels – 5.1 ± 2.4 ng/mL in the period from 1 to 5 years of follow-up. In several cases, outpatient examination revealed reasons for late conversion to proliferative signaling inhibitors in order to reduce CNI doses. The reason for conversion in 23 (1.7%) recipients was progressive nephropathy and early development of cardiac graft vasculopathy. By the end of year 5 of follow-up, the proportion of patients converted to EV increased to 8.6. The mean serum EV levels during the observation period were 3.8 ± 2.1 ng/mL. Despite daily administration of immunosuppressants, development of acute cellular and antibody-mediated rejection in this patient cohort cannot be ruled out. Outpatient detection of first-occurring cardiac rhythm disturbances, decreased left ventricular ejection fraction, as well as decreased tolerance to physical exertion, were a reason for hospitalization of recipients in order to rule out acute transplant rejection response.

Between January 2008 and December 2022, 5,274 endomyocardial biopsies were performed. Acute cellular rejection developing during the first year of follow-up was diagnosed in 841 (27.5%) recipients. According to the international classification, mild rejection (R1G) was diagnosed in 786 (25.7%) cases, moderate rejection (R2G) was diagnosed in 48 (1.57%) recipients, and severe (R3G) cardiac graft rejection was detected in 0.23% (n = 7) of cases. One year after HT, there was a decrease in the incidence of acute cellular graft rejection crises development, which was confirmed by the results of biopsy material examination. Thus, in the period from 1 year to five years, R1G rejection was diagnosed in 55 (2.48%) and R2G in 13 (0.59%) recipients. No severe degree of cellular rejection, according to endomyocardial biopsies, was revealed. The incidence of antibody-mediated rejection at different follow-up periods was 7.17%.

Annual hospitalization of heart transplant recipients to assess the coronary artery and diagnose cardiac graft vasculopathy is mandatory when monitoring this category of patients. The absence of innervation of the donor heart and clinical manifestations of angina makes it difficult for early detection of myocardial ischemia, which may lead to irreversible consequences.

Cardiac graft vasculopathy was diagnosed in 286 recipients. Percutaneous coronary intervention was required in 47% (n = 134) of cases at different follow-up periods. Indications for myocardial revascularization were coronary lesions with stenosis >70% with the possibility of balloon angioplasty with stenting. In the remaining 152 patients, graft vasculopathy was characterized by obliteration of the distal channel, which technically did not allow to perform myocardial revascularization. After one year of follow-up, cardiac graft vasculopathy was diagnosed in 2.3% of patients. After 3 and 5 years of follow-up, chronic graft rejection was diagnosed in 12% and 17% of recipients. The data obtained indicate that in the period from 3 to 5 years of follow-up, there is a tendency for graft vasculopathy to progress.

Among the heart transplant recipients observed, high blood pressure (HBP) was one of the modifiable risk factors for adverse events, including cardiac graft dysfunction. During the first year of outpatient follow-up, HBP of varying severity was diagnosed in 37.87% of heart transplant recipients. At year 5 of follow-up, the number of patients with HBP had increased to 48.65%; by year 9 of follow-up, the proportion of recipients suffering from HBP was 60.4%. Fig. 1 shows the dynamics of HBP detection depending on the period after the surgical intervention.

Angiotensin-converting enzyme (ACE) inhibitors, angiotensin receptor blockers (ARBs), slow calcium channel blockers (CCBs), diuretics (thiazide or loop diuretics, depending on the GFR) were prescribed as antihypertensive therapy. Drug doses were titrated individually depending on blood pressure fluctuation during the day. Fig. 2 shows the main groups of antihypertensive drugs prescribed for heart transplant recipients.

In our observation, the proportion of patients who required triple-drug antihypertensive therapy was 18% of recipients.

Post-transplant diabetes (PTDM) is a pathognomonic feature of the course of the long-term postoperative period and a risk factor for cardiovascular complications in patients with transplanted heart. Fig. 3 shows the frequency of detection of posttransplant diabetes in outpatient recipients.

All patients with PTDM (n = 298) received therapy to maintain normal blood sugar levels. Discussions on the importance of lifestyle modification and dietary adherence were also conducted on an outpatient basis. Of the 298 recipients with PTDM, 11.4% (n = 34) received insulin therapy; in the remaining 88.6%, maintenance of normal blood sugar levels was achieved by taking tablet forms of sugar-lowering drugs. Therapy effectiveness was evaluated by the level of glycated hemoglobin.

One of the aims of outpatient follow-up of heart transplant recipients is to assess renal function in order to rule out nephropathy against the background of long-term use

of CNIs. At the time of discharge from the hospital, the average rate of GFR was 89.11 ± 24.28 mL/min/1.73 m^2. At control outpatient visits during year 1 of follow-up, stage 3a chronic kidney disease (CKD) (GFR 53.15 ± 3.68) was diagnosed in 132 patients, stage 3b in 92 (GFR 39.07 ± 4.61), stage 4 in 48 (GFR 23.87 ± 3.87) and stage 5 was detected in 18 (GFR 8.3 ± 0.82) patients.

By the end of year 5 of follow-up, mean GFR was 74.92 ± 19.54 mL/min/1.73 m^2. Thus, after 5 years of follow-up, the proportion of patients with CKD increased

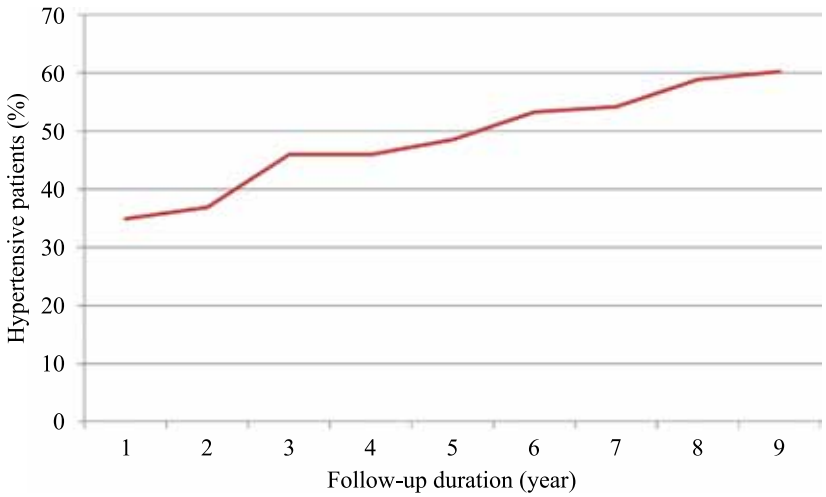

Fig. 1. Frequency of HBP detection depending on follow-up duration

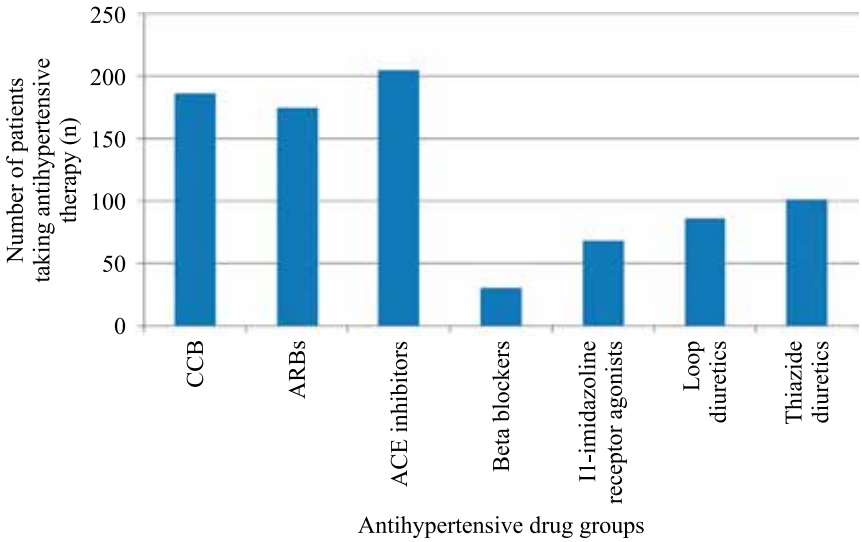

Fig. 2. Antihypertensive drug groups. CCB, slow calcium channel blockers; ARBs, angiotensin receptor blockers; ACE inhibitors, angiotensin-converting enzyme (ACE) inhibitors; I1-imidazoline receptor agonists

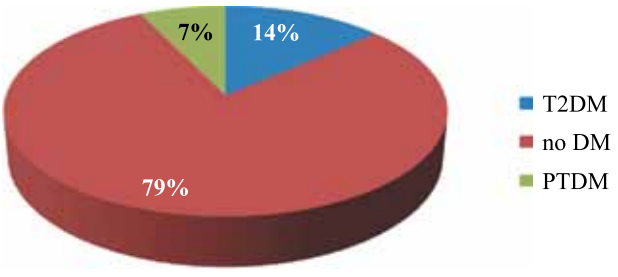

Fig. 3. Type 2 diabetes mellitus and post-transplant diabetes mellitus in heart transplant recipients. T2DM, type 2 diabetes mellitus; PTDM, post-transplant diabetes mellitus

with this patient cohort. Studies conducted in different periods of time at Shumakov Center have shown that complex therapy, including immunosuppressive therapy in combination with adjuvant therapy, significantly improves long-term survival in heart transplant recipients [4–6]. The personalized approach led to improvements in survival curves between time intervals 2007–2009, 2010–2012, 2013–2015, 2016–2018 and 1986–1991; 1992–1997, 1998–2003, and 2004–2006. However, since 2007, long-term survival rates have reached a plateau, and the median survival rate is 10.3 years, which requi-

and was distributed as follows: 260 recipients with stage 3a CKD (GFR 54.12 ± 4.75), 160 patients suffered from stage 3b CKD (GFR 38.45 ± 4.43), 68 recipients had stage 4 CKD (GFR 26.52 ± 2.84), end-stage CKD was diagnosed in 25 patients (GFR 8.1 ± 3.54). Twenty heart transplant recipients required long-term renal replacement therapy. After clinical and instrumental examination, 11 heart transplant recipients were included in the kidney transplant waiting list, seven of them underwent successful kidney transplantation within 6 months to one year.

When assessing the incidence of malignant tumors, it was found that at the follow-up period of 5–6 years after surgery, oncopathology was diagnosed in 61 recipients. The most common cancers were skin cancer (19.7%), lung cancer (16.4%), gastric cancer (16.4%), colorectal cancer (14.7%), oral cancer (9.8%), prostate cancer (6.6%), hepatocellular carcinoma (4.9%), thyroid cancer (4.9%) and others (6.6%).

Most recipients suffering from oncopathology were persons of working age from 40 to 65 years old.

When analyzing the causes of adverse events among outpatient recipients, it was shown that the main cause of mortality in the first year of follow-up was acute graft rejection. During the next 5 and 10 years of follow-up, most deaths were due to graft dysfunction on the background of coronary vasculopathy and oncologic diseases (see Table 2).

Survival analysis of heart transplant recipients, excluding in-hospital mortality, showed that survival at year 1, 5, and 10 of follow-up was 96.5%;, 88.0%, and 53.4%, respectively (see Fig. 4).

Median survival, excluding in-hospital mortality, is 10.5 years.

DISCUSSION

Results of this study have shown that outpatient follow-up of heart transplant recipients within the framework of personalized approach to medical care with the involvement of specialists from Shumakov Center and primary outpatient physicians allows for early correction of immunosuppressive therapy and detection of complications developing at different follow-up periods. The dual control model allows for professional consultations of patients at the transplant center. It also facilitates better professional training of local specialists working

Table 2

Causes of mortality at different follow-up periods after heart transplantation

Cause of death	1-year follow-up (n = 50)	5-year follow-up (n = 122)	≥10-year follow-up (n = 74)
Cardiac allograft vasculopathy	10 (4.06%)	45 (18.29%)	23 (9.35%)
Rejection	23 (9.35%)	23 (9.35%)	10 (4.06%)
Cancer	2 (0.81%)	16 (6.5%)	10 (4.06%)
Infection	6 (2.44%)	16 (6.5%)	18 (7.32%)
Multiple-organ failure	8 (3.25%)	11 (4.47%)	9 (3.66%)
Cerebrovascular complications	1 (0.41%)	4 (1.63%)	2 (0.81%)
Other causes	–	5 (2.03%)	2 (0.81%)

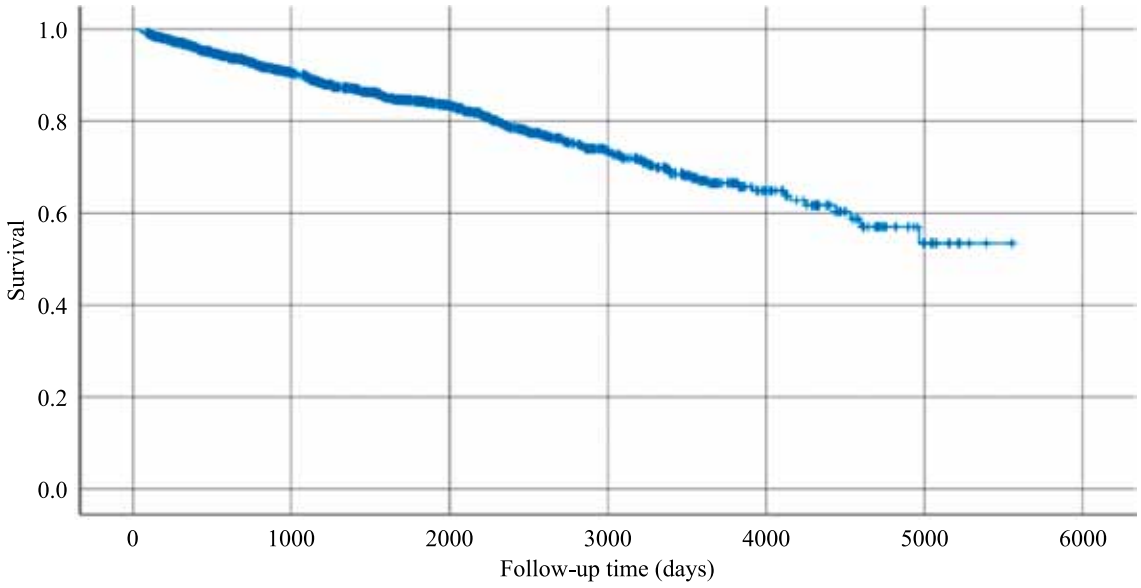

Fig. 4. Recipient survival

res further improvement and the development of new algorithms for outpatient care for heart transplant recipients, taking into account the increasing number of heart transplants performed annually [7].

CONCLUSION

The main objective of the Consultative and Diagnostic Department, Shumakov Center, is to implement a personalized approach to the monitoring and treatment of patients after HT. This includes individual immunosuppressive therapy regimens, laboratory and instrumental diagnostic methods that are aimed at early detection of complications and determination of further treatment tactics. We believe that this approach will improve survival and quality of life of heart transplant recipients in the long-term post-HT period.

The authors declare no conflict of interest.

REFERENCES

1. Transplantologiya: rukovodstvo dlya vrachej. Pod red. V.I. Shumakova. 2-e izd., ispr. i dop. M.: MIA, 2006; 544.
2. Transplantologiya: itogi i perspektivy. Tom XIII. 2021 god. Pod red. S.V. Gautier. M.–Tver': Triada, 2022; 416.
3. *Gautier SV, Shevchenko AO, Popcov VN.* Pacient s transplantirovannym serdcem. M.–Tver': Triada, 2014: 144.
4. *Shevchenko AO, Nikitina EA, Mozheiko NP, Tyunyaeva IYu, Koloskova NN.* Prevalence and predictors of hypertension in cardiac recipients. *Russian Journal of Transplantology and Artificial Organs*. 2017; 19 (3): 33–39. (In Russ.). https://doi.org/10.15825/1995-1191-2017-3-33-39.
5. *Poptsov VN, Zolotova EN.* Heart transplantation in diabetic recipients. *Russian Journal of Transplantology and Artificial Organs*. 2018; 20 (1): 120–126. (In Russ.). https://doi.org/10.15825/1995-1191-2018-1-120-126.
6. *Sakhovsky SA, Koloskova NN, Goncharova AYu, Mironkov BL.* Intravascular visualization methods in estimating vasculopathy of a transplanted heart. *Russian Journal of Transplantology and Artificial Organs*. 2019; 21 (1): 165–168. (In Russ.). https://doi.org/10.15825/1995-1191-2019-1-165-168.
7. *Koloskova NN.* Medicated therapy with transplanted heart recipients: … M.D. 14.01.24. M., 2020; 212.

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EFFECT OF TRYPSIN ON BIOCHEMICAL AND FUNCTIONAL PROPERTIES OF DECELLULARIZED PORCINE ARTICULAR CARTILAGE

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Objective: to study the effect of trypsin pretreatment in the porcine articular cartilage decellularization protocol on the ability to restore the biochemical composition and functional properties of the resulting finely dispersed tissue-specific scaffold when co-cultured with human adipose-derived stem cells (hADSCs). **Materials and methods.** Porcine articular cartilage was micronized to a maximum size of 250 μm. The resulting porcine articular cartilage microparticles (CMps) were treated with trypsin (0.05, 0.25, 0.50%) / EDTA solution at +37 °C for 24 hours. Then, the CMps were successively incubated for 24 hours in three surfactant solutions containing 0.1% sodium dodecyl sulfate and increasing concentration of Triton X-100 (1, 2, 3%) at room temperature and in DNase I solution at +37 °C for 48 hours. The degree of change in the biochemical composition and the ability of decellularized CMps (DCMps) scaffolds within cell-engineered constructs (CECs) to support hADSC adhesion and proliferation, as well as their potential ability to exert a stimulatory regenerative effect, were then assessed. DNA, glycosaminoglycans (GAGs) and collagen content in the DCMps and CECs were examined. The morphology of the samples was examined using histological and immunohistochemistry staining. **Results.** Histological analysis showed that there were no cells and detritus in the DCMp samples. Pretreatment of CMps using a solution with the lowest content of trypsin (0.05%) / EDTA in the samples retained 5.14 ± 0.87 ng/mg DNA in the samples, while GAG content decreased to 5.34 ± 0.9 μg/mg and collagen to 154 ± 34 μg/mg. By day 28 of CEC cultivation, adherent cells had produced their own extracellular matrix (ECM) containing GAGs and collagen. The amount of DNA in it was 6.30 ± 0.11 μg/CEC and that of GAGs was 19.36 ± 0.73 μg/CEC. **Conclusion.** Pretreatment with trypsin allows achieving uniformly complete decellularized CMps. At the same time, onset of changes in the ECM composition indicates a decrease in the ability of hADSCs to synthesize GAGs and type II collagen during co-culturing with DCMps. The increased proliferative activity of adherent hADSCs, as well as the tissue specificity of the DCMp scaffold will allow further research towards a hydrogel matrix capable of enhancing the specific and stimulating regenerative potential when co-cultured with cells of the same phenotype.

Keywords: cartilage tissue, decellularization, trypsin, mesenchymal stromal cells, tissue engineering.

INTRODUCTION

Minimally invasive intra-articular injection of CECs, which consist of resorbable biocompatible matrices (scaffolds, carriers), loaded with stem or tissue-specific cells and bioactive molecules, represents a promising therapeutic solution that could restore the structure and functions of damaged cartilage. Decellularized tissue matrices look encouraging, they are capable not only of keeping the cells in the area around cartilage damage, but also of providing them with the necessary conditions for their vital activity.

Decellularization is the process by which cells are completely destroyed and cellular material is removed from a tissue or organ under certain influences. An important task of decellularization is to preserve ECM components as much as possible, which allows the tissue-specific scaffold to maintain cell adhesion, proliferation and differentiation [1].

Currently, tissue-specific scaffolds are obtained from decellularized whole organs (liver, kidneys, heart, lungs, pancreas) [2–6] or from decellularized organ microfragments [7, 8] using physical, chemical and enzymatic processing methods [9]. Quite often, several processing methods are used concurrently. For example, when decellularizing cartilage tissue, freeze-thaw cycles, treatment with DNase I [10] and supercritical carbon dioxide ($scCO_2$) are added to standard surfactant treatment. This facilitates diffusion of decellularizing agents into the ECM volume [11] and, thus, provides more effective cell lysis [12], reduces time and increases homogeneity of tissue processing.

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One method of decellularization of organs and tissues is to treat them with chelating agents such as ethylenediaminetetraacetic acid (EDTA). EDTA promotes cell dissociation from ECM proteins by binding metal ions [13, 14]. Since chelating agents alone are not sufficient to remove cells even with intensive agitation [15], they are usually used in combination with enzymes, including trypsin [16, 17].

Trypsin is commonly used as an enzymatic decellularization agent to detach cells from ECM structural proteins, destroy tissue ultrastructure and improve diffusion into the volume of subsequent decellularizing agents [16, 17]. The degree of removal of cells and ECM components under the action of trypsin depends on the incubation period. Complete decellularization by trypsin alone may require a long incubation period (at least 24 hours), even for non-cellular tissues [18]. Note that proteins have limited resistance to trypsin cleavage [19], so prolonged trypsin exposure even in 0.03–0.05% concentration can significantly reduce the concentration of ECM biomolecules (GAGs, collagen, elastin), disrupt its structure and change mechanical properties [20–22].

So, a combination of two agents – trypsin and EDTA – improves the decellularization effectiveness at the initial stage. At the same time, to minimize the negative effect of trypsin on ECM proteins, trypsin concentration is reduced by adding other methods to the decellularization process, such as treatment of tissue with surfactant or DNase. This is especially relevant for complete removal of cell nuclei from dense tissues such as cartilage [23].

Although pretreatment with trypsin has been described in a number of cartilage-decellularization protocols [24, 25], these studies are limited to analysis of biochemical and mechanical properties of the decellularized matrix, as well as evaluation of its cytotoxicity in vitro and functional efficiency in vivo. Moreover, the effect of a significant decrease in GAG and collagen content in the decellularized cartilage tissue on the ability of adherent hADSCs to synthesize GAG and type II collagen when co-cultured was not investigated.

The objective of this work was to study the effect of including trypsin pretreatment in the porcine articular cartilage decellularization protocol on the ability to restore the biochemical composition and functional properties of the resulting finely dispersed tissue-specific scaffold in the adhesion of hADSCs during their co-culturing.

MATERIALS AND METHODS

Object of study

The object of study, articular cartilage of the hips and knees of pigs, was obtained from agro-industrial holding Promagro based in Stary Oskol, after the slaughter of healthy animals. After transportation in chilled form (+4 °C), the cartilage was removed from articular surfaces with a scalpel, sliced into fragments no larger than 5 × 5 × 5 mm in size, frozen at –20 °C and stored at this temperature until cryogenic grinding began. CMps were obtained by cryogenic grinding method using cryogenic grinder CryoMill (Retsch, Germany).

Particle sizing

Size distribution of CMps in the suspension was determined by laser diffraction using the flow cell of the SALD-7101 laser diffraction analyzer (Shimadzu, Japan). A refractive index of 1.35 was used to measure the size of the microparticles. Glycerol was chosen as the dispersion medium. Data were processed using WingSald II software (Shimadzu, Japan).

Decellularization modes

A 100 mg sample of CMps was placed in a 2,500 units/mg trypsin solution (Sigma-Aldrich, USA) at 0.05%, 0.25%, and 0.50% concentrations with 0.53 mM EDTA (Sigma-Aldrich, USA) and incubated at +37 °C and periodic stirring on a magnetic stirrer (3 times daily for 1 hour at 200 rpm speed) for 24 hours.

The CMps were then treated in three changes of phosphate-buffered saline pH = 7.4 (PanEco, Russia) containing 0.1% sodium dodecyl sulfate (SDS) (Sigma-Aldrich, USA) and increasing concentration of Triton X-100 (Sigma-Aldrich, USA), at room temperature and periodic stirring:

1. Solution containing 1% Triton X-100 + 0.1% SDS, 24 hours.
2. Solution containing 2% Triton X-100 + 0.1% SDS, 24 hours.
3. Solution containing 3% Triton X-100 + 0.1% SDS, 24 hours.

To achieve complete decellularization, the CMps were additionally incubated in DNase I solution (CyStorLab, Russia) for 48 hours at +37 °C.

An aqueous solution of an antibiotic (ampicillin, 20 μg/ml) and an antimycotic (amphotericin B, 2 μg/ml) was used to wash off decellularizing agents from the DCMps.

The washed DCMps matrix samples were sterilized by γ-irradiation with a 1.5 Mrad dose.

DNA quantification

DNA was isolated using the DNeasy Blood&Tissue Kit (QIAGEN, Germany) according to the manufacturer's instructions. Double-stranded DNA was detected using a Quant-iT Picogreen dsDNA Assay Kit and dsDNA Reagents (ThermoFisherScientific, USA) according to the manufacturer's instructions. Further analysis was performed using a Spark 10M microplate spectrofluorometer (Tecan Trading, Switzerland) at 520 nm wavelength.

Quantification of GAGs

To analyze GAG content, the samples were preincubated in papain solution (Sigma-Aldrich, USA) at +65 °C

for 12 hours. Cationic dye 1,9-Dimethyl-Methylene blue zinc chloride double salt (DMMB) (Sigma-Aldrich, USA) was used for GAG measurement. Staining was performed in a 96-well plate: 20 μL of lysate and 200 μL of the working dye solution were added to the well, and then the GAG content was determined on a Spark 10M microplate spectrofluorometer (Tecan Trading, Switzerland) at 525 nm wavelength.

Collagen quantification

Collagen content was determined using Sircol Soluble Collagen Assay kit (Biocolor, UK) in original tissue samples and in DCMps. For collagen extraction, all samples were lysed in 0.01 M HCl solution containing 1 mg/mL pepsin (Sigma-Aldrich, USA) for 12 hours at room temperature. The resulting lysates were treated with reagents from the kit according to the manufacturer's instructions. The optical density in each sample was determined in 96-well plates at 556 nm wavelength on a Spark 10M microplate spectrofluorometer (Tecan Trading, Switzerland).

Study of the functional properties of DCMps matrix scaffold

The functional efficiency of the tissue-specific DCMps matrix is to maintain the adhesion, proliferation and functional activity of tissue-specific cells in vitro. We investigated the ability of DCMps and hADSCs to form CECs containing GAGs and collagen when co-cultured. The source of hADSCs was the subcutaneous adipose tissue of a healthy donor obtained with informed voluntary consent.

Each CEC included 5 mg of sterile DCMps and 5×10^5 hADSCs obtained under aseptic conditions. The matrix was populated with cells in tubes with complete cell culture medium (CCCM) on a MultiBio 3D orbital shaker (Biosan, Latvia) and then incubated under standard conditions for 3 days. The CCCM contained DMEM/F12 (PanEco, Russia) (1 : 1) with addition of 10% fetal bovine serum (HyClone, USA), 1% antibiotic-antimycotic (Gibco, USA) and 2 mM L-glutamine (PanEco, Russia). CCCM was then replaced with chondrogenic differentiation medium that included DMEM HG (Gibco, USA), 10% ITS+ (Corning, USA), 1% sodium pyruvate (Gibco, USA), 0.25% ascorbate-2-phosphate (Sigma-Aldrich, USA), 0.0001% dexamethasone (Sigma-Aldrich, USA), 0.002% transforming growth factor beta 1 (TGF-β1) (PeproTech, USA), and 1% antibiotic-antimycotic (Gibco, USA), and cultured for 28 days. The medium was replaced every second day. Third passage cells were used in the experiment.

Viability of hADSCs in CECs was assessed by in vivo staining using the LIVE/DEAD Viability/Cytotoxicity Kit (Thermo Fisher Scientific, USA) and a Leica DMi8 Thunder inverted microscope (Leica Microsystems, Germany). Morphology of the samples was examined using histological and immunohistochemistry staining.

Histological and immunohistochemical study

The samples were fixed in 10% formalin solution, washed in running water and dehydrated in alcohols of ascending concentration, incubated in ethanol and chloroform, then in chloroform, and embedded in paraffin. Sections were deparaffinized, rehydrated, and stained with DAPI, hematoxylin and eosin, alcian blue, and Masson's trichrome. Immunohistochemical study for type II collagen was performed using Novocastra Lyophilized Rabbit Polyclonal Collagen Type II; for visualization we used Novocastra Concentrated Peroxidase Detection System (Leica Biosystems, Germany). Analysis and photography of the obtained preparations were performed using an inverted Nikon EclipseTi microscope (Nikon, Japan).

RESULTS AND DISCUSSION

Fig. 1 shows the measurement results of 5 DCMp samples. The microparticle size did not exceed 220 μm,

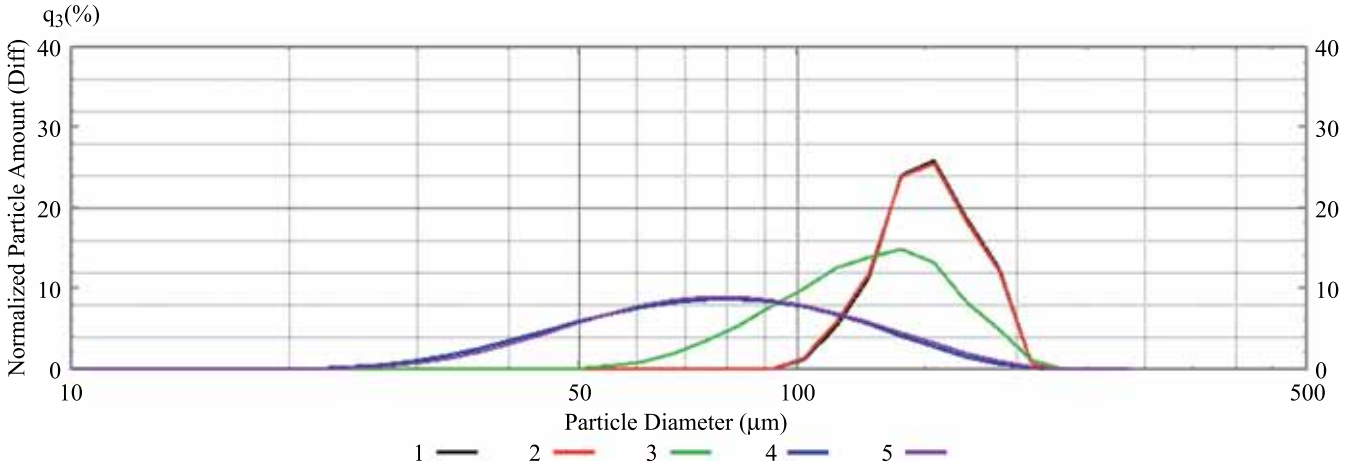

Fig. 1. Microparticle size distribution in suspension

thereby making it possible to develop a minimally invasive DCMps matrix-based injectable form of CEC for use.

Previously, we showed [10] that the use of surfactant alone does not provide an effective degree of CMp decellularization. It was suggested that introduction of a stage featuring pretreatment of CMps with trypsin/EDTA solution followed by incubation of samples in surfactant and DNase solutions would increase the completeness of decellularization.

In the present study, no cells were detected in DCMps during histological analysis, regardless of the trypsin concentration used (Fig. 2). Nuclear material and cell detritus were also not visualized, indicating uniform decellularization of CMps (Figs. 2, d, i, m). The absence of nuclear material in all DCMps matrices (Fig. 2, h, l, p), in contrast to the initial tissue state (after cryodestruction) (Fig. 2, d), was confirmed when samples were stained with DAPI fluorescent dye.

When subjected to Masson's trichrome staining, the ECM of the original tissue was homogeneously stained exclusively blue due to the presence of collagen [26]. At the same time, when DCMps matrix was stained in the same way, metachromasia was detected, which increased with an increase in trypsin concentration (Fig. 2, f, j, n), indicating biochemical changes in the decellularized ECM.

The native ECM of articular cartilage was stained intensively with alcian blue for GAGs (Fig. 2, c), in contrast to DCMps matrix samples, in which no GAGs were detected by the qualitative method (Fig. 2, g, k, o).

Data from histological analysis confirmed the high efficiency of decellularization of articular cartilage tissue – absence of cells and cellular detritus, regardless of trypsin concentration in the range from 0.05% to 0.50%. Therefore, taking into account that enzymatic methods of decellularization, in particular trypsin, reduce the residual number of GAGs in decellularized tissues [27], we performed further studies only with DCMps matrix that was treated with the minimum trypsin concentration

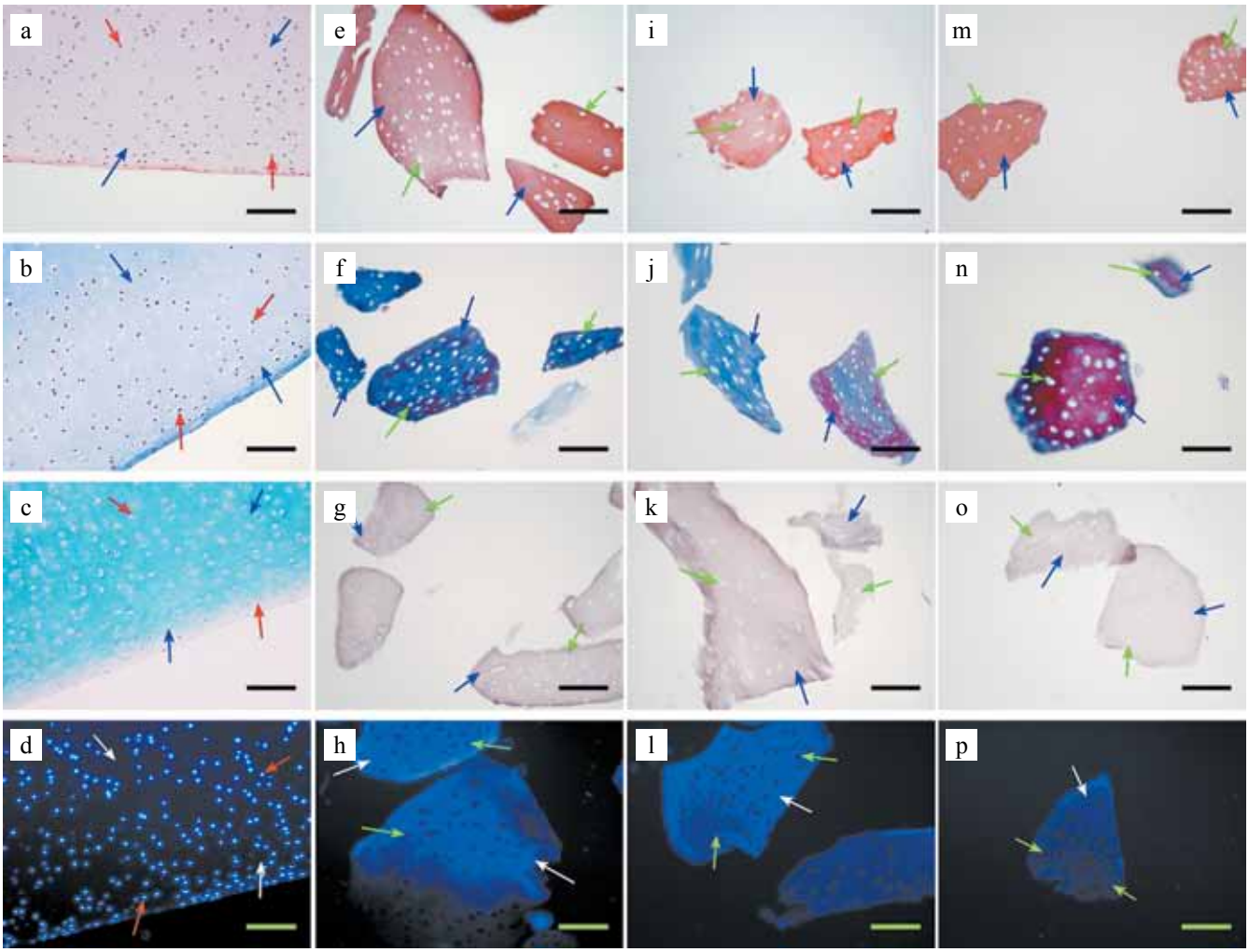

Fig. 2. Histological examination of original and decellularized cartilage tissue. a, b, c, d, original tissue; e, f, g, h, decellularization using 0.05% trypsin; i, j, k, l, decellularization using 0.25% trypsin; m, n, o, p, decellularization using 0.50% trypsin. a, e, i, m, H&E stain; b, f, j, n, Masson's trichrome stain; c, g, k, o, Alcian blue stain; d, h, l, p, DAPI stain. Blue and white arrows indicate microparticles, red arrows show cells, and green arrows indicate empty lacunas. Scale bar = 100 μm

(0.05%), allowing to minimize the negative effect of trypsin on the ECM composition.

Another indicator of the effectiveness of the chosen decellularization method is the results of determining the concentration of residual DNA in DCMps matrix samples compared to the original articular cartilage tissue. After pretreatment with trypsin (0.05% concentration)/EDTA, the DNA content in DCMps matrix decreased significantly from 366.85 ± 53.03 ng/mg of tissue in the original samples to 5.14 ± 0.87 ng/mg of tissue after decellularization.

We have previously explored the effect of including various additional treatment steps in the CMp decellularization protocol. It was found that the optimal, in terms of removing DNA, cells, and cellular detritus, as well as preserving the biochemical composition of DCMps, is a protocol that includes, in addition to surfactant and DNAase, additional ultrasound (US) treatment. With this method of CMp treatment, the residual DNA amount was 1.8 ± 0.6 ng/mg of tissue [28].

DNA residual amount in DCMps matrices that were additionally treated with both trypsin and US [28] was less than 50 ng/mg of tissue, which is the minimum criterion for satisfying the decellularization goal [13]. In addition, visible nuclear material on histological sections of DCMps matrices stained with H&E and DAPI was absent, indicating the low immunogenicity of DCMps matrices.

In the present study, the sharp decrease (almost complete absence) of GAGs in DCMps was confirmed by quantification results: GAG content was found to be 5.34 ± 0.9 μg/mg and 154 ± 22 μg/mg in DCMps and original tissue, respectively.

The content of fibrillar collagen after cartilage decellularization with trypsin also decreased to 154 ± 34 μg/mg compared to its amount in the original cartilage tissue – 508 ± 103 μg/mg. This indicates significant enzymatic hydrolysis of collagen in DCMps when treated with trypsin for a day at +37 °C [29]. It can be assumed that reducing the DCMps incubation time and/or temperature will increase preservation of fibrillar collagen structure in DCMps.

Application of additional US treatment of microparticles allowed us earlier [12] to minimize the loss of GAGs and fibrillar collagen in DCMps matrix: their content was 58 ± 12 μg/mg and 417 ± 47 μg/mg, respectively.

Thus, introduction of enzymatic pretreatment with trypsin at a minimum concentration of 0.05% in the cartilage decellularization process allows us to remove cells and their fragments, as well as to significantly reduce DNA content in DCMps to 5.14 ± 0.87 ng/mg (by more than 90%), which was impossible to achieve when using surfactant without additional treatment methods [10]. However, simultaneously with the decrease in DNA in DCMps, there was a decrease in concentration of fibrillar collagen (by 70%) and GAG (by 97%), indicating a significant destructive effect of trypsin on ECM. We observed a considerable decrease in GAG when using other articular cartilage decellularization protocols as well [28].

The next stage of the work was to investigate the functional properties of the DCMps matrix. In this regard, we carried out an experiment on creation of CECs consisting of DCMps and adherent hADSCs, followed by assessment of the viability of these cells in the process of co-culturing, as well as performing biochemical and histological studies of CECs.

In a previous study, we showed that the immunophenotypic profile of marker expression in cells isolated from adipose tissue met the International Society for Cell & Gene Therapy criteria and confirmed that these cells are multipotent mesenchymal stem cells. The primary cell culture was characterized by a high level of expression of CD29, CD44, CD49b, CD73 and CD90, while no expression of CD34, CD45 or HLA-DR was observed in the culture [30].

Our in vivo fluorescence microscopy showed that by day 21 of hADSC culturing, there was higher cell count on the matrix surface (Fig. 3). We observed the fusion of DCMps and the formation of a single conglomerate. In the main mass of green-stained live cells, dead cells with a red color were also determined.

During histological examination in CECs, we observed DCMps microparticles connected by adherent and newly formed ECM cells into a single conglomerate, as well as pronounced cell proliferation (Fig. 4).

Areas of cell necrosis accompanied by the formation of karyorrhexis products were observed in the central zone by day 14 of cultivation. This is down to the insufficient supply of nutrients deep into the CECs. At the same time, the number of hyperchromic nuclei increased with increasing duration of cultivation, which indicates a disturbance in the structure of the cell nucleus and, accordingly, an increase in the count of cells that are in a state of degradation. In the surface zone of CECs, we observed natural destructive cellular processes only by day 21 of cultivation. When subjected to Masson's trichrome stain, uniformly distributed collagen fibers were seen. When subjected to alcian blue stain, the ECM containing GAG was visualized. The uniformity of ECM staining increased with increasing cultivation time, indicating an increase in GAG production by adherent and newly formed cells.

Immunohistochemical staining of ECM for type II collagen at day 28 of CECs culture revealed positive staining (Fig. 5). However, the staining was not intense, indicating that the adherent and newly formed cells poorly produced the main type of collagen of the articular cartilage.

Biochemical examination of CECs at day 28 of cultivation included determination of DNA and GAG con-

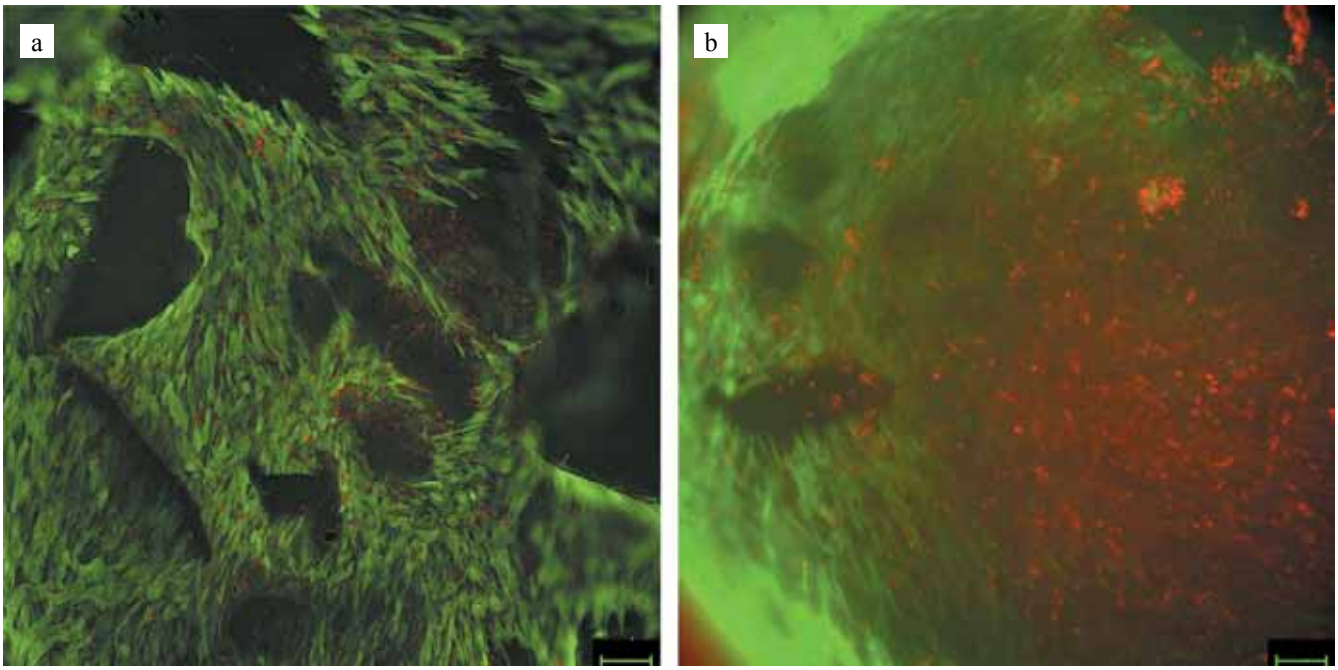

Fig. 3. Study of cell viability in CECs. a, day 14; b, day 21. Live/Dead stain. Scale bar = 100 μm

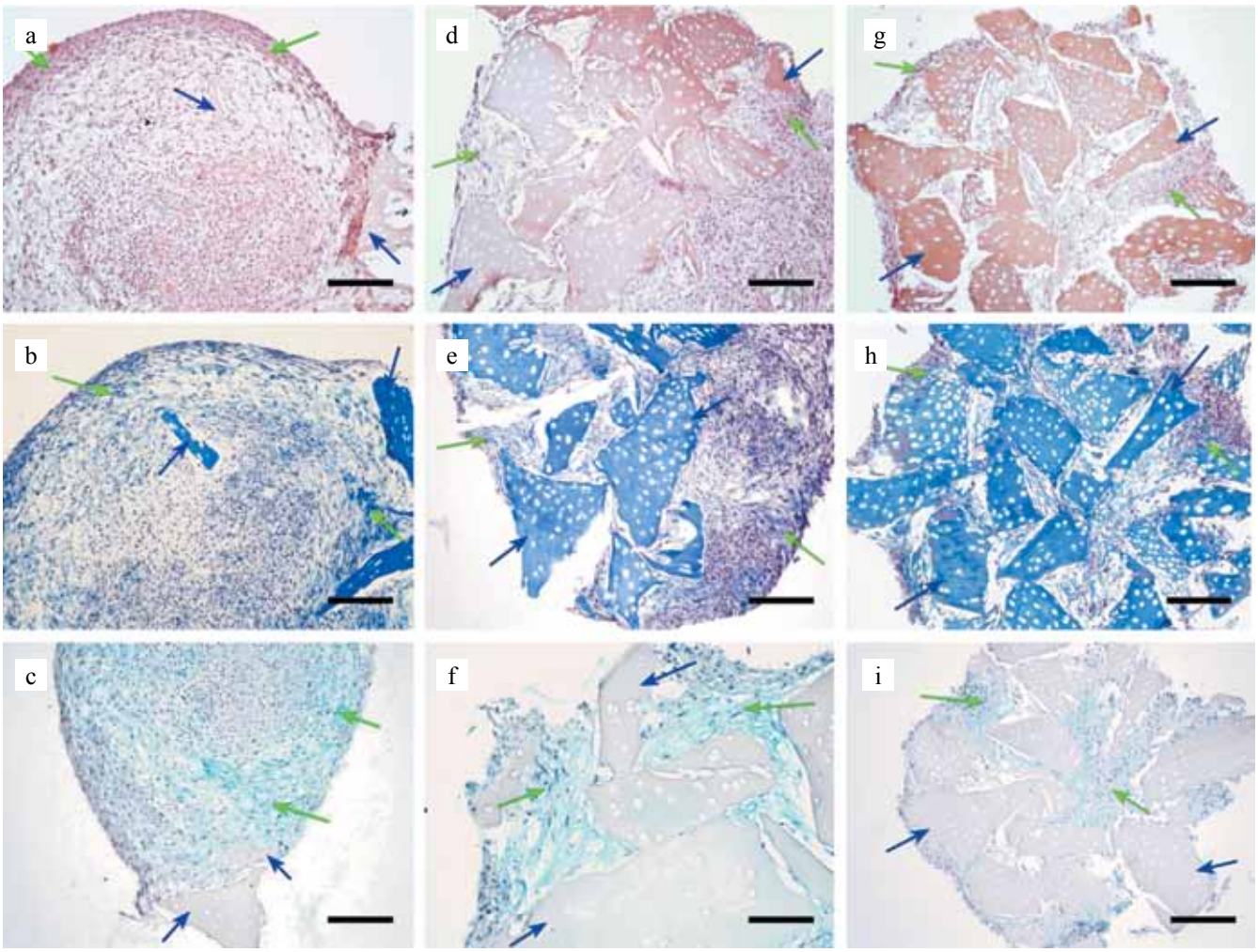

Fig. 4. Histological examination of CEC. a, b, c, day 14 of cultivation; d, e, f, day 21 of cultivation; g, h, i, day 28 of cultivation; a, d, g, H&E stain; b, e, h, Masson's trichrome stain; c, f, i, Alcian blue stain. Blue arrows indicate decellularized porcine articular cartilage microparticles, green arrows indicate ECM produced by adherent and newly formed cells. Scale bar = 100 μm

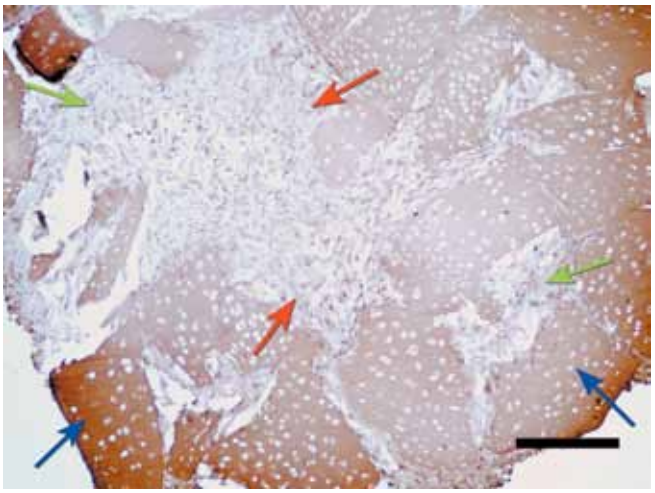

Fig. 5. Immunohistochemical study of CECs for type II collagen. On day 28 of cultivation, CECs contained little type II collagen. Blue arrows indicate decellularized porcine articular cartilage microparticles, green arrows indicate ECM produced by adherent and newly formed cells, red arrows indicate type II collagen produced by cells. Scale bar = 100 μm

centration in them. The amount of DNA was found to increase from 5.14 ± 0.87 ng/mg in DCMps to 6.30 ± 0.11 μg/CEC, and the amount of GAG also increased from 5.34 ± 0.9 μg/mg in DCMps to 19.36 ± 0.73 μg/CEC. Based on these data, the level of GAG production per unit DNA was calculated to be 3.07 ± 0.61 GAG, μg/DNA, μg.

Earlier [28], we have conducted a comparative study of the efficiency of several CMps decellularization protocols with respect to the completeness of cell and gene material removal while preserving the main ECM components as much as possible, and we evaluated the functional properties of CECs. It was found that when hADSCs were co-cultured with DCMps matrix decellularized with by pretreatment with 0.05% trypsin/EDTA, the DNA amount at day 28, reflecting the appearance of new cells, was higher than when using protocols with other additional treatments (freeze/thaw cycles, $scCO_2$, US). However, the number of GAGs produced by adherent cells on DCMps matrices was lower, indicating a negative effect of trypsin/EDTA treatment of ECM. GAG production per unit DNA was also minimal (3.07 ± 0.61 GAG, μg/DNA, μg) compared with other protocols (freeze/thaw: 13.6 ± 2.2 GAG, μg/DNA, μg, $scCO_2$: 7.1 ± 1.2 GAG, μg/DNA, μg, US: 8.4 ± 1.6 GAG, μg/DNA, μg).

These results of culturing hADSCs in CECs confirmed that when introduced trypsin/EDTA pretreatment to the decellularization protocol, DCMps matrix more effectively supports cell adhesion and growth of cell proliferation compared to other protocols but contributes to the reduction of cellular GAG production in ECM [28].

Similar data were obtained in works [31, 32, 33], where the effect of adding trypsin treatment to the bovine and porcine cartilage decellularization process on the properties of the resulting matrix was studied. Additional trypsin treatment resulted in a significant decrease in GAG content, but the decellularized tissue retained its mechanical and biocompatibility properties. In addition, decellularized costal cartilage produced a biosafe and mechanically strong matrix with great potential for clinical application in rhinoplasty.

Trypsin was also used for decellularization of other types of specialized tissues, such as porcine myocardial tissue [34], where the growth and proliferation of endothelial cells on the decellularized matrix was shown with the loss of ECM components and absence of cardiac markers expression by adherent cells.

Results obtained and analysis of literature data show that trypsin has a destructive effect on the biochemical composition of ECM during tissue decellularization – it reduces GAG and collagen content. At the same time, it was found that partial loss of the main ECM components does not negatively affect the use of trypsin-decellularized tissues as biocompatible scaffold implants in areas like rhinoplasty and tracheoplasty [32, 33].

Earlier in the development of tissue engineering and regenerative medicine technologies for the treatment of pathological changes in cartilage tissue, we have pointed out two possible ways of CEC application [35]: stimulation of physiological regeneration of damaged tissue structures and partial or complete temporary replacement of the function of damaged tissue structures.

We have shown that DCMps-based CEC (a tissue-specific ECM mimetic), obtained by a decellularization protocol that includes several treatments [28], is able to form a tissue equivalent of cartilage tissue more effectively than the hydrogel ECM mimetic, a biopolymer-based microheterogeneous collagen-containing hydrogel (BMCH). At the same time, the stimulating effect of DCMps-based CECs on the processes of physiological regeneration was lower than that of BMCH-based CECs [36].

Summarizing the results of our studies, we assume that the detected increase in proliferative activity of cells when trypsin pretreatment is involved in CMps decellularization, as well as the preserved tissue-specificity of the DCMps matrix, will allow to continue research towards creating a hydrogel matrix form with a higher specific (chondrogenic) and prolonged stimulating regenerative effect.

CONCLUSION

The results indicate that trypsin pretreatment allows achieving uniform and complete decellularization of CMps. However, changes in the biochemical composition arising in the obtained DCMps matrices reduce the ability of adherent hADSCs and newly formed cells, when co-cultured in CECs, to synthesize GAG, type II collagen and form ECM, the cell activity environment.

Meanwhile, when trypsin/EDTA pretreatment is included in the CMps decellularization protocol, the proliferative activity of adherent hADSCs increases. The increased proliferative activity as well as the tissue-specificity of the DCMps matrix will allow further research towards creating a hydrogel matrix form that can increase the specific and stimulatory regenerative potential of ECM when co-cultured with cells of the same phenotype.

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The authors declare no conflict of interest.

REFERENCES

1. *Cramer MC, Badylak SF*. Extracellular matrix-based biomaterials and their influence upon cell behavior. *Annals of Biomedical Engineering*. 2020; 48 (7): 2132–2153. doi: 10.1007/s10439-019-02408-9. PMID: 31741227.
2. *Jambar Nooshin B, Tayebi T, Babajani A, Khani MM, Niknejad H*. Effects of different perfusing routes through the portal vein, hepatic vein, and biliary duct on whole rat liver decellularization. *Cell journal*. 2023; 25 (1): 35–44. doi: 10.22074/cellj.2022.557600.1081. PMID: 36680482.
3. *Hsu CY, Chi PL, Chen HY, Ou SH, Chou KJ, Fang HC et al.* Kidney bioengineering by using decellularized kidney scaffold and renal progenitor cells. *Tissue and Cell*. 2022; 74: 101699. doi: 10.1016/j.tice.2021.101699. PMID: 34891081.
4. *Tang-Quan KR, Mehta NA, Sampaio LC, Taylor DA*. Whole cardiac tissue bioscaffolds. *Advances in Experimental Medicine and Biology*. 2018; 1098: 85–114. doi: 10.1007/978-3-319-97421-7_5. PMID: 30238367.
5. *Bölükbas DA, De Santis MM, Alsafadi HN, Doryab A, Wagner DE*. The preparation of decellularized mouse lung matrix scaffolds for analysis of lung regenerative cell potential. *Methods in Molecular Biology*. 2019; 1940: 275–295. doi: 10.1007/978-1-4939-9086-3_20. PMID: 30788833.
6. *Berger C, Bjørlykke Y, Hahn L, Mühlemann M, Kress S, Walles H et al.* Matrix decoded – a pancreatic extracellular matrix with organ specific cues guiding human ipsc differentiation. *Biomaterials*. 2020; 244: 119766. doi: 10.1016/j.biomaterials.2020.119766. PMID: 32199284.
7. *Khajavi M, Hashemi M, Kalalinia F*. Recent advances in optimization of liver decellularization procedures used for liver regeneration. *Life Sciences*. 2021; 281: 119801. doi: 10.1016/j.lfs.2021.119801. PMID: 34229008.
8. *Sevastianov VI, Ponomareva AS, Baranova NV, Kirsanova LA, Basok YB, Nemets EA et al.* Decellularization of human pancreatic fragments with pronounced signs of structural changes. *International Journal of Molecular Sciences*. 2023; 24 (1): 119. doi: 10.3390/ijms24010119. PMID: 36613557.
9. *Qian H, He L, Ye Z, Wei Z, Ao J*. Decellularized matrix for repairing intervertebral disc degeneration: fabrication methods, applications and animal models. *Materials Today Bio*. 2022; 18: 100523. doi: 10.1016/j.mtbio.2022.100523. PMID: 36590980.
10. *Basok YuB, Kirillova AD, Grigoriev AM, Kirsanova LA, Nemets EA, Sevastianov VI.* Fabrication of microdispersed tissue-specific decellularized matrix from porcine articular cartilage. *Perspektivnye materialy*. 2020; 5: 51–60. [In Russ, English abstract]. doi: 10.30791/1028-978x-2020-5-51-60.
11. *Nemets EA, Lazhko AE, Basok YuB, Kirsanova LA, Kirillova AD, Sevastianov VI.* Features of obtaining a tissue-specific matrix from decellularized porcine cartilage. *Sverhkriticheskie flyuidy: teoriya i praktika*. 2020; 15 (2): 3–13. [In Russ, English abstract]. doi: 10.34984/SCFTP.2020.15.2.001.
12. *Keane TJ, Swinehart IT, Badylak SF*. Methods of tissue decellularization used for preparation of biologic scaffolds and *in vivo* relevance. *Methods*. 2015; 84: 25–34. doi: 10.1016/j.ymeth.2015.03.005. PMID: 25791470.
13. *Crapo PM, Gilbert TW, Badylak SF*. An overview of tissue and whole organ decellularization processes. *Biomaterials*. 2011; 32 (12): 3233–3243. doi: 10.1016/j.biomaterials.2011.01.057. PMID: 21296410.
14. *Gilpin A, Yang Y*. Decellularization strategies for regenerative medicine: from processing techniques to applications. *BioMed Research International*. 2017; 2017: 9831534. doi: 10.1155/2017/9831534. PMID: 28540307.
15. *Gailit J, Ruoslahti E*. Regulation of the fibronectin receptor affinity by divalent cations. *Journal of Biological Chemistry*. 1988; 263 (26): 12927–12932. doi: 10.1016/s0021-9258(18)37650-6. PMID: 2458338.
16. *Maurer P, Hohenester E*. Structural and functional aspects of calcium binding in extracellular matrix proteins. *Matrix Biology*. 1997; 15 (8–9): 569–580. doi: 10.1016/S0945-053X(97)90033-0. PMID: 9138289.
17. *Hopkinson A, Shanmuganathan VA, Gray T, Yeung AM, Lowe J, James DK et al.* Optimization of amniotic membrane (am) denuding for tissue engineering. *Tissue Engineering – Part C: Methods*. 2008; 14 (4): 371–381. doi: 10.1089/ten.tec.2008.0315. PMID: 18821842.
18. *Schenke-Layland K, Vasilevski O, Opitz F, König K, Riemann I, Halbhuber KJ et al.* Impact of decellularization of xenogeneic tissue on extracellular matrix integrity for tissue engineering of heart valves. *Journal of Structural Biology*. 2003; 143 (3): 201–208. doi: 10.1016/j.jsb.2003.08.002. PMID: 14572475.
19. *Waldrop FS, Puchtler H, Meloan SN, Younker TD*. Histochemical investigations of different types of collagen. *Acta histochemica Supplementband*. 1980; 21: 21–31. PMID: 6808564.
20. *Lin CH, Kao YC, Ma H, Tsay RY*. An investigation on the correlation between the mechanical property change and the alterations in composition and microstructure of a porcine vascular tissue underwent trypsin-based decellularization treatment. *Journal of the Mechanical Behavior of Biomedical Materials*. 2018; 86: 199–207. doi: 10.1016/j.jmbbm.2018.06.029. PMID: 29986294.
21. *Grauss RW, Hazekamp MG, Oppenhuizen F, Van Munsteren CJ, Gittenberger-De Groot AC, DeRuiter MC*. Histological evaluation of decellularised porcine aortic valves: matrix changes due to different decellularisation

methods. *European Journal of Cardio-thoracic Surgery*. 2005; 27 (4): 566–571. doi: 10.1016/j.ejcts.2004.12.052. PMID: 15784352.

22. *Merna N, Robertson C, La A, George SC*. Optical imaging predicts mechanical properties during decellularization of cardiac tissue. *Tissue Engineering – Part C: Methods*. 2013; 19 (10): 802–809. doi: 10.1089/ten.tec.2012.0720. PMID: 23469868.
23. *Giraldo-Gomez DM, García-López SJ, Tamay-de-Dios L, Sánchez-Sánchez R, Villalba-Caloca J, Sotres-Vega A et al.* Fast cyclical-decellularized trachea as a natural 3d scaffold for organ engineering. *Materials Science and Engineering C*. 2019; 105: 110142. doi: 10.1016/j.msec.2019.110142. PMID: 31546345.
24. *Rahman S, Griffin M, Naik A, Szarko M, Butler PEM*. Optimising the decellularization of human elastic cartilage with trypsin for future use in ear reconstruction. *Scientific Reports*. 2018; 8 (1): 3097. doi: 10.1038/s41598-018-20592-x. PMID: 29449572.
25. *Vernice NA, Berri N, Bender RJ, Dong X, Spector JA*. Production of a low-cost, off-the-shelf, decellularized cartilage xenograft for tissue regeneration. *Annals of Plastic Surgery*. 2022; 88 (3): S296–S301. doi: 10.1097/SAP.0000000000003185. PMID: 35513335.
26. *Dmitrieva EG, Khatsko SL, Yakimov AA*. Staining method for coronary arteries. *Sibirskij nauchnyj medicinskij zhurnal*. 2022; 42 (3): 47–51. [In Russ, English abstract]. doi: 10.18699/SSMJ20220305.
27. *Gilbert T, Sellaro T, Badylak S*. Decellularization of tissues and organs. *Biomaterials*. 2006; 27 (19): 3675–3683. doi: 10.1016/j.biomaterials.2006.02.014. PMID: 16519932.
28. *Sevastianov VI, Basok YB, Grigoriev AM, Nemets EA, Kirillova AD, Kirsanova LA et al.* Decellularization of cartilage microparticles: effects of temperature, supercritical carbon dioxide and ultrasound on biochemical, mechanical, and biological properties. *Journal of Biomedical Materials Research – Part A*. 2023; 111 (4): 543–555. doi: 10.1002/jbm.a.37474. PMID: 36478378.
29. *Semenycheva LL, Egorihina MN, Chasova VO, Valetova NB, Mitin AV, Kuznetsova YuL*. Efficacy of pancreatin and trypsin proteases in enzymatic hydrolysis of collagen. *Bulletin of the South Ural State University series "Chemistry"*. 2020; 12 (1): 66–75. [In Russ, English abstract]. doi: 10.14529/chem200108.
30. *Tsvetkova AV, Vakhrushev IV, Basok YB, Grigor'ev AM, Kirsanova LA, Lupatov AY et al.* Chondrogeneic potential of msc from different sources in spheroid culture. *Bulletin of Experimental Biology and Medicine*. 2021; 170 (4): 528–536. doi: 10.1007/s10517-021-05101-x. PMID: 33725253.
31. *Ghassemi T, Saghatoleslami N, Mahdavi-Shahri N, Matin MM, Gheshlaghi R, Moradi A*. A comparison study of different decellularization treatments on bovine articular cartilage. *Journal of Tissue Engineering and Regenerative Medicine*. 2019; 13 (10): 1861–1871. doi: 10.1002/term.2936. PMID: 31314950.
32. *Lin S, He Y, Tao M, Wang A, Ao Q*. Fabrication and evaluation of an optimized xenogenic decellularized costal cartilage graft: preclinical studies of a novel biocompatible prosthesis for rhinoplasty. *Regenerative Biomaterials*. 2021; 8 (6): rbab052. doi: 10.1093/rb/rbab052.
33. *Giraldo-Gomez DM, Leon-Mancilla B, Del Prado-Audelo ML, Sotres-Vega A, Villalba-Caloca J, Garciadiego-Cazares D et al.* Trypsin as enhancement in cyclical tracheal decellularization: morphological and biophysical characterization. *Materials Science and Engineering C*. 2016. doi: 10.1016/j.msec.2015.10.094. PMID: 26652450.
34. *Perea-Gil I, Uriarte JJ, Prat-Vidal C, Gálvez-Montón C, Roura S, Llucià-Valldeperas A et al. In vitro* comparative study of two decellularization protocols in search of an optimal myocardial scaffold for recellularization. *American Journal of Translational Research*. 2015; 7 (3): 558–573. PMID: 26045895.
35. *Basok YuB, Sevastianov VI*. Tissue engineering and regenerative medicine technologies in the treatment of articular cartilage defects. *Russian Journal of Transplantology and Artificial Organs*. 2016; 18 (4): 102–122. [In Russ, English abstract]. doi: 10.15825/1995-1191-2016-4-102-122.
36. *Sevastianov VI, Basok YuB, Kirsanova LA, Grigoriev AM, Kirillova AD, Nemets EA et al.* A comparison of the capacity of mesenchymal stromal cells for cartilage regeneration depending on collagen-based injectable biomimetic scaffold type. *Life*. 2021; 11 (8): 756. doi: 10.3390/life11080756.

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TECHNIQUE FOR REDUCING THE SURGICAL POROSITY OF SMALL-DIAMETER VASCULAR GRAFTS

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High surgical porosity (SP) is one of the causes of significant blood loss, as well as hematoma formation. So, reducing the SP of small-diameter vascular grafts (VGs) is a crucial task. The **objective** of this work was to develop a technology for the formation of polycaprolactone (PCL)-based small-diameter VGs with a bioactive coating with reduced SP. **Materials and methods.** Porous VGs with an inner diameter of 3 mm were fabricated by electrospinning from 5% PCL solution with addition of 5–30% gelatin (PCL/G) on an NANON-01A unit (MECC C^O, Japan). Bioactive coating was applied by sequential incubation of VGs in solutions of bovine serum albumin, heparin and platelet lysate with fixation in a glutaric aldehyde solution. The surface structure and mechanical properties of the samples were investigated. Functional properties of the bioactive VGs were evaluated in relation to their interaction with cell cultures *in vitro*. **Results.** It was found that introduction of gelatin into the working solution reduces SP from 30.4 ± 1.5 mL/(cm^2·min) to 2.8 ± 0.5 ml/(cm^2·min). It was shown that at a PCL/gelatin ratio of 9 : 1, the outer and inner sides of the bioactive VGs samples are characterized by surface uniformity (no defects), mechanical properties close to blood vessels of the same diameter (Young's modulus 6.7 ± 2.1 MPa, tensile strength 26.7 ± 4.9 N and elongation to break 423 ± 80%) and ability to support adhesion and proliferation of human umbilical vein endothelial cell line, EA.hy926. **Conclusion.** Introduction of 10% gelatin content (by the polymer weight) into PCL solution reduces the SP of small-diameter VGs, leads to uniformity in their inner and outer surface, improvement in their mechanical properties without reducing their ability to support adhesion and proliferation of vascular endothelial cells.

Keywords: small-diameter vascular grafts, polycaprolactone, gelatin, surgical porosity, mechanical properties, endothelial cells.

Surgical porosity (SP) is one of the most important characteristics of vascular grafts (VGs). GOST 31514-2012 [1] defines it as water permeability, the amount of water going through 1 cm^2 of VG wall area during 1 minute at 120 mm Hg pressure. High permeability of the implant promotes the formation of hematomas, which, when organized, cause fibrosis and reduced prosthesis lumen [2, 3]. Water permeability >50 mL/(cm^{-2}·min^{-1}) is a criterion that determines the need for additional efforts to reduce SP [4, 5].

A study of textile VGs made of polyethylene terephthalate showed that for the pore size (20 to 100 μm), there is a positive correlation ($R^2 > 0.9$) between water permeability and blood loss, but blood loss is about 10 times less than water permeability [6]. This is down to the higher viscosity of blood plasma and the presence of formed elements in it.

The simplest method to reduce surgical porosity is to impregnate the finished vascular graft with a natural sealant. Whole blood is most often used as a sealant [1]. The method is called "preclotting" (from the word clot, a blood clot). Immediately before implantation, the implant is impregnated with fresh autologous blood containing no anticoagulants and incubated at 37 °C for a time interval sufficient to ensure fibrin formation [7]. This method exists up to the present, including for electrospinning-derived VGs [8]. In addition, fibrin glue [9], cross-linked hydrogels based on proteins (albumin, collagen, gelatin, etc. [10–13]), chondroitin sulfate [14], silk fibroin [15], sodium alginate [16], dextran derivatives [17], and chitosan [18], are used as a fibrin source to reduce the SP of high-porosity VGs.

One of the significant problems of hydrogel coatings for reducing VG porosity is their rather high resorption

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rate, which leads to the need for additional crosslinking [19–22]. Crosslinking agents (dialdehydes, polyepoxy compounds, isocyanates, etc.) are toxic substances that are difficult to completely remove from the coating volume. Besides, additional neutralization of unreacted reactive groups and potentially toxic derivatives formed by the interaction of crosslinking agents with proteins is required.

A distinctive feature of the electrospinning method is the possibility to obtain highly porous materials from fibers with diameters varying in a wide range from hundreds of nanometers to tens of micrometers. By varying the formation parameters (polymer solution concentration, nature of solvent, voltage between electrodes, humidity, temperature, etc.), it is possible to obtain highly porous materials with different fiber structure, pore size and surface morphology [22–25].

Previously obtained VGs, made of pure polycaprolactone, had a SP close to the maximum permissible value [26]. The objective of this study was to optimize the VG formation technology in the form of 3 mm diameter tubes with reduced water permeability.

MATERIALS AND METHODS

Fabrication of vascular grafts

Tubular VG specimens with an inner diameter of 3 mm were made by electrospinning from 5% (w/w) solution of polycaprolactone (PCL, MM 80000, Sigma-Aldrich, USA), as well as from PCL with gelatin addition (Sigma-Aldrich, USA) at a concentration of 5–30% (by the polymer weight) in hexafluoroisopropanol (PIM-INVEST, Russia), on electrospinning unit NANON-01A (MECC C^{O}, Japan) at a 25 kV voltage between the electrodes, 4 mL/h solution feed rate, 100 mm distance to the collector, 1000 rpm rotation speed of the substrate rod, using a 18 G needle. After the end of the solution application process, the obtained samples were dried in a thermostat at 37 °C for 2 hours, followed by vacuuming to remove traces of the solvent at 10–20 mmHg residual pressure and 37 °C for 24 hours.

Application of bioactive coating on the vascular graft surface

To form a bioactive coating, the VGs were incubated in 1 mg/mL bovine serum albumin solution (SPE PanEco, Russia) for 1.5–2 hours at 37 °C, then treated with aqueous 1 mg/mL heparin solution (Sigma-Aldrich, USA) for 1.5–2 hours at 37 °C. The coating was fixed with 1% glutaraldehyde solution for 18 hours at room temperature, then re-treated with 1 mg/mL heparin solution for 1.5–2 hours at 37 °C. Between the stages and at the end of the modification procedure, the vascular graft was washed three times in 100 mL of distilled water. The obtained heparinized sample was dried at 37 °C with subsequent vacuuming at room temperature and 10–20 mm Hg residual pressure, and sterilized by gamma radiation at a 1.5 Mrad dose.

The required volume of human platelet lysate solution (hPL, Renam, Russia) was obtained by diluting it in a 1 : 9 ratio with Hanks' Balanced Salt Solution containing no Ca^{2+} and Mg^{2+} ions (HBSS, Gibco® by Life Technologies™). The hPL solution was sterilized by filtration through a membrane filter with a pore diameter of 0.22 μm. Sterile heparinized VG samples were treated with hPL solution under aseptic conditions for 1 hour at 37 °C immediately before the experiment.

Surface morphology of vascular grafts

The surface structure of the VG samples was analyzed on a scanning electron microscope (SEM) JSM-6360LA (JEOL, Japan) at an accelerating voltage of 5 kV and magnifications ×100 and ×500. To create a conductive coating, gold was sputtered on a JFC-1600 (JEOL, Japan) for 40 seconds at a 5–7 mA constant current.

Stress-strain behavior of vascular grafts

Mechanical tests of VG samples were conducted on tensile tester Shimadzu EZ Test EZ-SX (Shimadzu Corporation, Japan) with TrapeziumX software, version 1.2.6, at a tensile speed of 5 mm/min.

The following stress-strain properties of the samples were recorded in both longitudinal and transverse directions: elongation at break expressed as a percentage of the original sample size, tensile strength expressed in N, and Young's modulus expressed in MPa characterizing the ability of the test sample to stretch and compress.

Cultivation of human umbilical vein endothelial cell line, EA.hy926, on the vascular graft surface

The functional properties of the VG samples were evaluated by interaction with human umbilical vein endothelial cell line, EA.hy926 (endothelial cells), from the American Type Culture Collection (ATCC). All studies were performed under aseptic conditions. Before use, the endothelial cells were stored in liquid nitrogen at –196 °C. After thawing, the cells were seeded into 25 cm^2 or 75 cm^2 standard culture vials (CELLSTAR® Greiner Bio-One, Germany) and cultured in complete growth medium DMEM with high glucose content (4.5 g/L, DMEM high glucose with HEPES, PanEco, Russia) supplemented with 10% fetal bovine serum (ETS, HyClone,

USA), antibiotic and antimycotic Anti-Anti (Gibco®, Life Technologies Corporation, USA) and 2 mM glutamine (PanEco, Russia) in a CO_2 incubator under standard conditions: 37 °C, in a humidified atmosphere containing (5 ± 1)% CO_2. Before the experiment, cells were removed from the surface of culture plastic using dissociation reagent TrypLE™ Express Enzyme (Gibco®, Life Technologies Corporation, USA) and a suspension with the required cell concentration was prepared. The initial endothelial cell count in the suspension was determined using an automated cell counter (TC20™ Automated Cell Counter, BIORAD, Singapore) with simultaneous determination of viability by trypan blue dye exclusion (BIORAD, #145-0013, Singapore).

The studied sterile samples of unmodified and modified VGs were pre-cut lengthwise, straightened, placed on the bottom of a flat-bottomed 24-well culture plate (CELLSTAR® Greiner Bio-One, Germany) with the inner side up and fixed with sterile silicone rings and seeded under aseptic conditions. The initial seeding density of the endothelial cells on the tested samples was 5×10^4 cells/cm^2. After seeding, the plates with samples were cultured in a CO_2 incubator under standard conditions for a specified time interval.

Assessment of metabolic activity and cell count

The metabolic activity of endothelial cells was recorded using PrestoBlue™ HS Cell Viability Reagent (Invitrogen™, Thermo Fisher Scientific, USA) according to the protocol recommended by the manufacturer. 10% PrestoBlue™ HS Cell Viability Reagent was added to wells containing the test samples and a cell-free control sample (cell-free complete growth medium), after which the plate was incubated for 4 hours at 37 °C in a humidified atmosphere containing (5 ± 1)% CO_2. Changes in absorbance of the medium were recorded using a Spark 10M microplate reader (Tecan, Austria) with Spark Control™ Magellan V1.2.20 software at 570 nm and 600 nm wavelengths. The percentage of reduced PrestoBlue™ characterizing the metabolic activity of the cells was then calculated according to formula (1):

$$\frac{117.216 \cdot A_{570\ Samp} - 80.586 \cdot A_{600\ Samp}}{117.216 \cdot A^0_{570} - 80.586 \cdot A^0_{600}} \times 100\%, \quad (1)$$

where: 117.216 and 80.586 are the molar extinction coefficients for the oxidized form of PrestoBlue™ Vital Reagent at 600 nm and 570 nm wavelengths, respectively; 155.677 and 14.652 are the molar extinction coefficients for the reduced form of PrestoBlue™ Vital Reagent at 570 nm and 600 nm wavelengths, respectively; $A_{570\ Samp}$ and $A_{600\ Samp}$ are the absorbance of the test sample at 570 nm and 600 nm wavelengths, respectively; A'_{570} and A'_{600} are the absorbance of the cell-free control sample at 570 nm and 600 nm wavelengths, respectively.

The number of the EA.hy926 endothelial cell line on the VG surface was estimated using calibration curves linear in semi-logarithmic coordinates up to a cell concentration of 0.8×10^5. To construct the calibration curve, cells were seeded into flat-bottomed 24-well culture plates (CELLSTAR® Greiner Bio-One, Germany) at a seeding density of 1–20 × 10^4 cells/cm^2. After 24 hours, PrestoBlue™ Vital Reagent was added to wells containing the required number of cells and a cell-free control sample, the plate was incubated for 3 hours at 37 °C in a humidified atmosphere containing (5 ± 1)% CO_2 and the change in media uptake was recorded. The percentage of recovered PrestoBlue™ determined by formula (1) was plotted on the graph on the y-axis, and the corresponding number of cells was plotted on the x-axis.

Statistical processing

Quantitative and statistical processing of the obtained data was performed using Microsoft Excel 2019. All results are presented as mean value ± standard deviation. Differences were considered reliable at $p < 0.05$.

RESULTS AND DISCUSSION

The results of the study of the effect of the amount of applied polymer and the concentration of introduced gelatin (G) on the surgical porosity of VGs are presented in Fig. 1.

The minimum SP of gelatin-free VGs made of pure PCL is 30.4 ± 1.5 mL/(cm^2·min) and is achieved when 2 mL of 5% solution is applied [26]. As can be seen from Fig. 1, in the case of application of 2 mL of PCL/G-BASED solution, regardless of the concentration of gelatin added to the PCL solution, the surgical porosity is minimal, 1.8 ± 0.1 mL/(cm^2·min).

When the amount of applied solution is reduced to 1 mL, a similar effect is achieved only when the gelatin concentration is increased to 20% or more.

Figs. 2 and 3 illustrate the effect of gelatin concentration added to PCL on the surface structure of 3 mm diameter VGs.

As can be seen from Figs. 2 and 3, regardless of the concentration of introduced gelatin, it leads to a decrease in the diameter of fibers and an increase in the density of their packing both from the inside and outside. This is the reason for the decrease in the SP of the vascular graft. PCL/gelatin samples with 5% and 15% gelatin concentration have partially porous internal structure (Fig. 2) with inclusions of extensive areas formed by soldered filaments, and at gelatin concentration of 20%

the inner surface of VGs looks monolithic with a small number of pores on the surface.

In the case of gelatin concentration of 10% and 30% (Fig. 2), a highly porous structure formed by individual submicron-sized filaments is preserved on the inner side of PCL/G. The sample with 10% gelatin is slightly more preferable due to minimal deformations as a result of less adhesion to the surface of the electrode rods.

On the outer side (Fig. 3), PCL/G-based VGs show a more porous surface structure compared to the inner side (Fig. 2). Moreover, while at a gelatin concentration of 20%, the inner surface of VGs looks almost monolithic (Fig. 2), the outer surface shows a pronounced porous structure with many open pores. Simultaneously with the increase in porosity on the outer surface of all the examined VGs, except for the sample containing 10% gelatin, traces of pronounced mechanical deformation as a result of separation of PCL/G from the substrate are observed.

Thus, the addition of gelatin to PCL at 10% concentration (by polymer weight) is optimal in terms of formation of VGs with highly porous structure and minimal deformation of both internal and external surfaces (Fig. 4).

Table shows the experimental results characterizing the effect of gelatin concentration on the physical and mechanical characteristics of VGs of different compositions.

As can be seen from Table, addition of gelatin to PCL leads to formation of more durable VGs, which is necessary for a product functioning under constant physical stress. At the same time, the effect of gelatin addition on elongation at break is insignificant. Moreover, the presence of gelatin increases the Young's modulus,

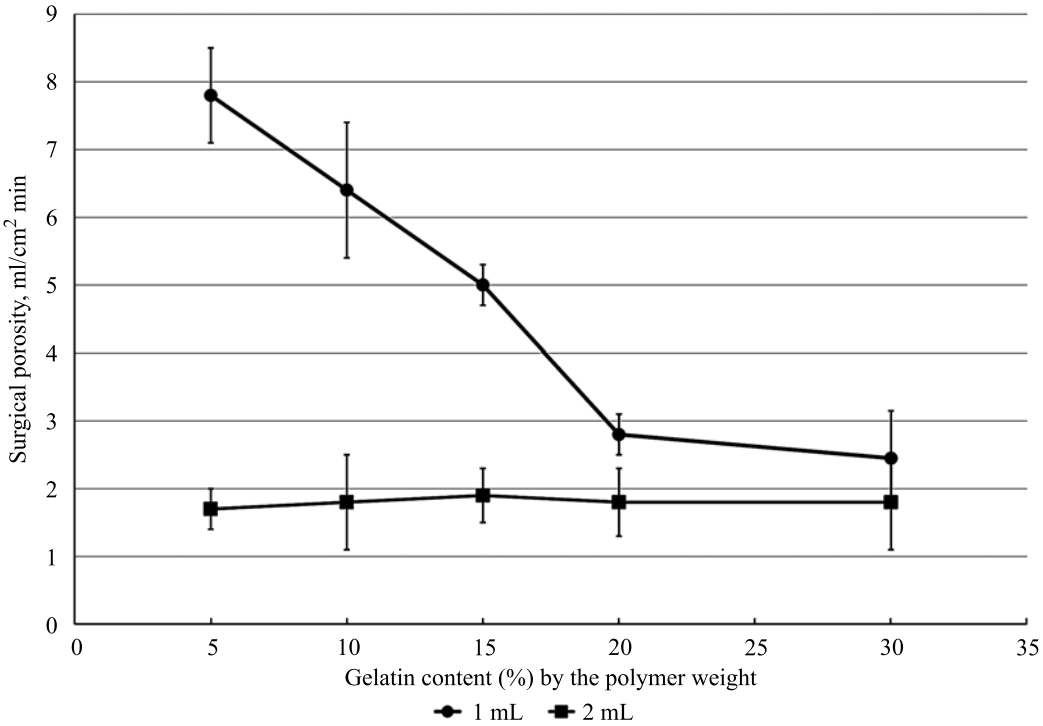


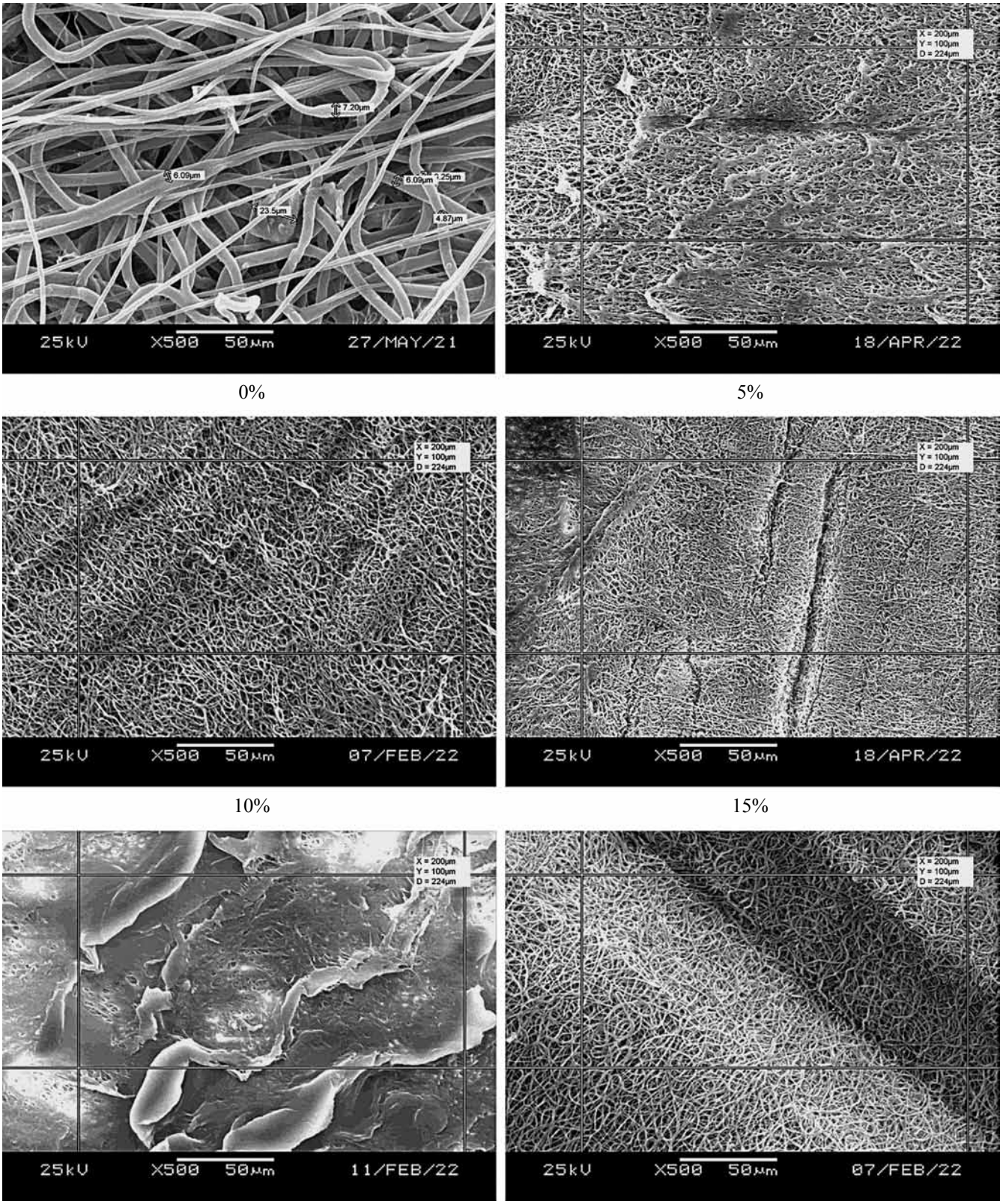
Fig. 1. Effect of the content of gelatin added to PCL and volume of the solution used on the surgical porosity of PCL/G-based VGs. Diameter 3 mm, solution flow rate 4 mL/hour

Table

Effect of the content of gelatin on the physical and mechanical characteristics of PCL/G-based VGs. Diameter 3 mm, volume 2 mL, solution flow rate 4 mL/hour

Gelatin content (%)	Young's modulus (MPa)	Tensile strength (N)	Elongation at break (%)
0	5.5 ± 1.1	10.9 ± 1.6	477 ± 38
5	11.3 ± 2.1	22.0 ± 4.7	441 ± 48
10	6.7 ± 0.7	26.7 ± 4.9	423 ± 80
15	10.7 ± 3.8	27.7 ± 3.4	432 ± 57
20	10.1 ± 4.7	26.7 ± 7.5	440 ± 129
30	11.7 ± 3.1	23.3 ± 2.9	448 ± 34

which is not desirable since the obtained values exceed those typical for natural human arterial blood vessels of the same diameter [27]. Among VGs with reduced SP due to the presence of gelatin, the variant with 10% gelatin (PCL/G10) is the most promising from the point of view of forming tissue-engineered constructs of small-diameter blood vessels, since it demonstrates increased strength and minimum Young's modulus that differ only



0% 5%

10% 15%

20% 30%

Fig. 2. Effect of gelatin content on the microstructure of the inner surface of PCL/G-based VGs. Diameter 3 mm, volume 2 mL, solution flow rate 4 mL/hour

slightly from those obtained in the case of VGs obtained from pure PCL.

The study of interaction of PCL/G10-based VGs, modified with bioactive coating with the culture of human umbilical vein endothelial cell line, EA.hy926, showed (Fig. 5) that the cells actively adhere to the tested surface (point 24 hour), and that they almost double in number after 168 hours of cultivation.

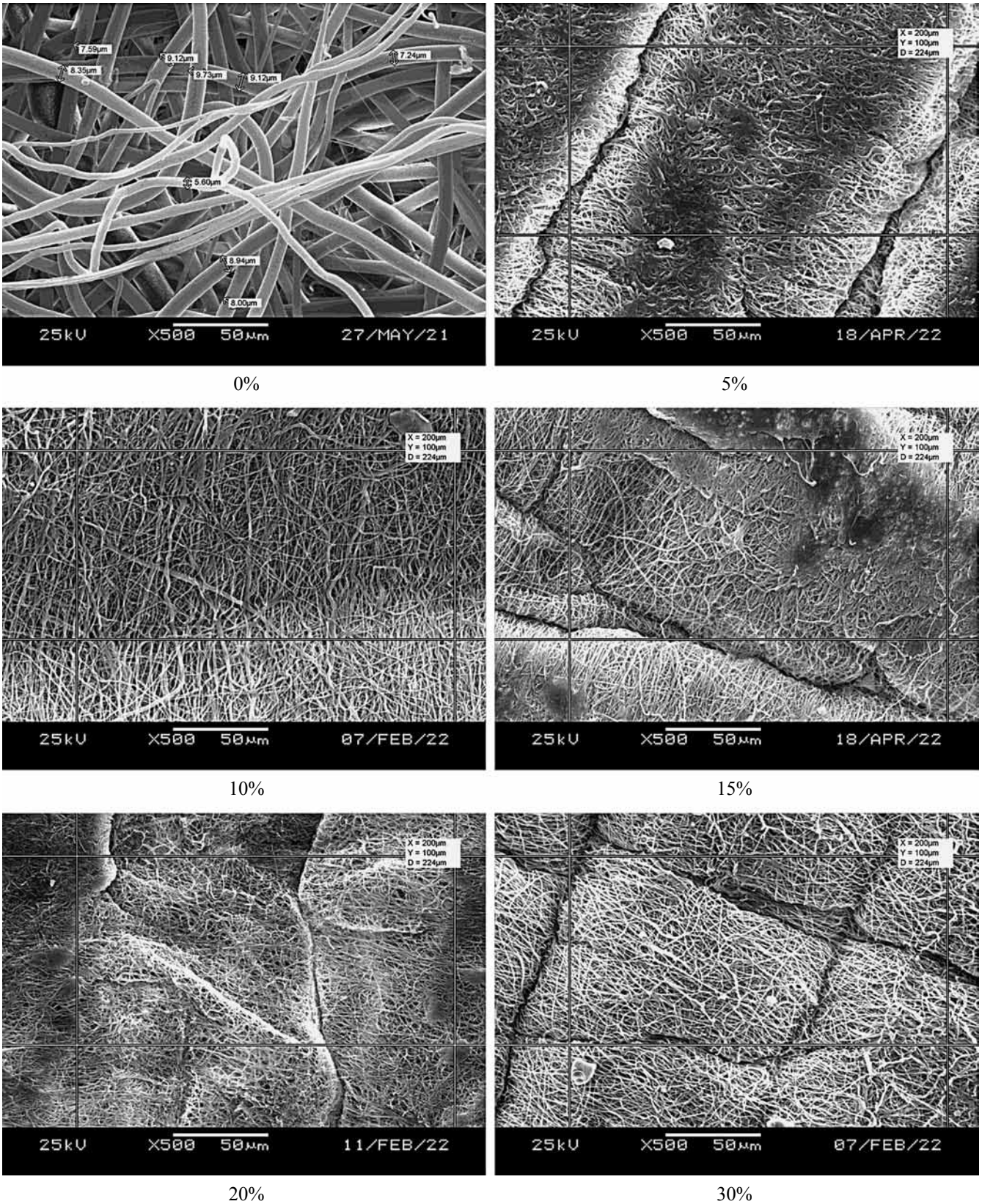
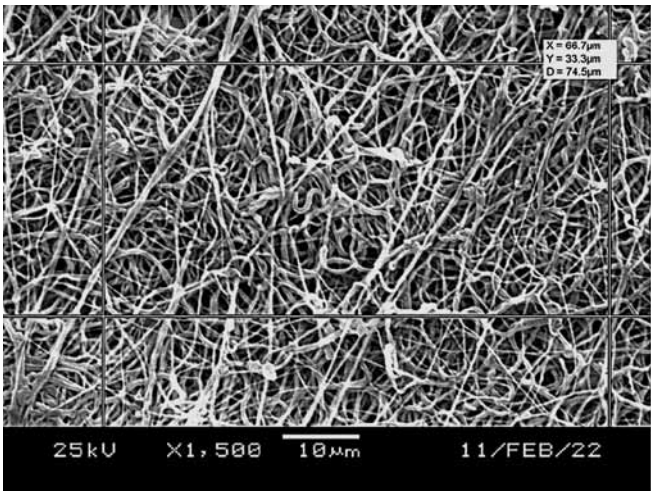
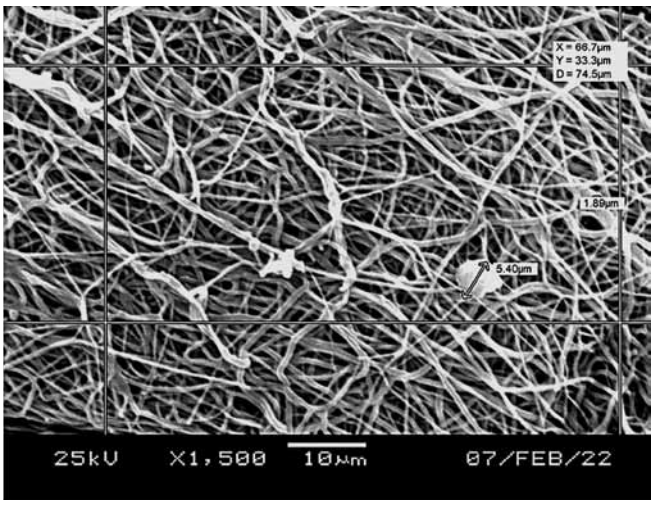


Fig. 3. Effect of gelatin content on the microstructure of the outer surface of PCL/G-based VGs. Diameter 3 mm, volume 2 mL, solution flow rate 4 mL/hour



Inner side



Outer side

Fig. 4. Structure of the surface of PCL/G-based VGs. Diameter 3 mm, gelatin content 10%, volume 2 mL, solution flow rate 4 mL/hour

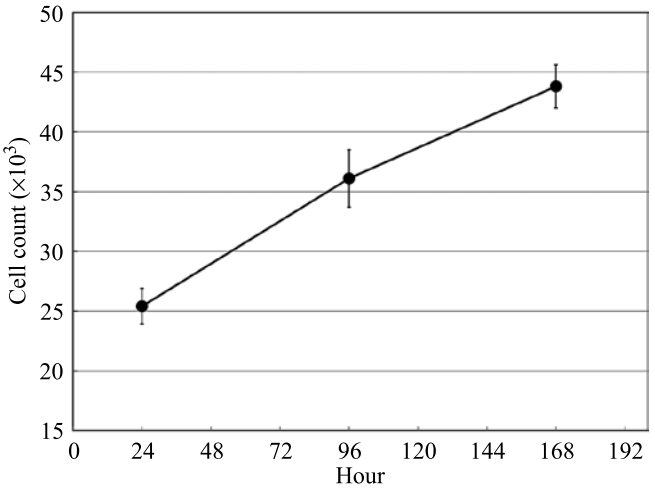


Fig. 5. Proliferation of human umbilical vein endothelial cell line, EA.hy926, on the inner surface of bioactive-coated PCL/G10-based VGs. Initial seeding density of 5×10^4 cells/cm^2

CONCLUSION

Addition of gelatin to PCL at 10% concentration (by polymer weight) is effective in terms of reducing the SP of small-diameter VGs, it provides the necessary stress-strain properties and minimal graft deformation from the inner and outer sides. The ability of bioactive coated VGs to support adhesion and proliferation of vascular endothelial cells has been confirmed. The next step is the study of the hemocompatibility and functional properties of the developed small-diameter bioactive VG sample *in vivo*.

The authors declare no conflict of interest.

REFERENCES

1. Blood vessel prostheses. General technical requirements. Test methods: GOST 31514-2012. Date of introduction 01.01.2015. M.: Standartinform, 2015.
2. *Szentivanyi A, Chakradeo T, Zernetsch H, Glasmacher B.* Electrospun cellular microenvironments: understanding controlled release and scaffold structure. *Adv Drug Deliv Rev*. 2011; 63: 209–220.
3. *Novikova SP, Salokhedinova RR, Loseva SV, Nikolashina LN, Levkina AYu*. Analysis of physico-mechanical and structural characteristics of vascular prostheses. *Thoracic and cardiovascular surgery.* 2012; 54: 27–33.
4. *Wesolowski SA, Fries CC, Karlson KE, De Bakey M, Sawyer PN.* Porosity: primary determinant of ultimate fate of synthetic vascular grafts. *Surgery*. 1961; 50: 91–96.
5. *Lebedev LV, Plotnik LL, Smirnov AD*. Protezy krovenosnykh sosudov. L.: Medicina, 1981; 192.
6. *Guan G, Yu C, Fang X, Guidoin R, King MW, Wang H, Wang L.* Exploration into practical significance of integral water permeability of textile vascular grafts. *J Appl Biomater Funct Mater.* 2021; 19: 22808000211014007. doi: 10.1177/22808000211014007.
7. *Yates SG, Barros D'Sa AA, Berger K, Fernandez LG, Wood SJ, Rittenhouse EA et al.* The preclotting of porous arterial prostheses. *Ann Surg.* 1978; 188: 611–622.
8. *Joseph J, Domenico Bruno V, Sulaiman N, Ward A, Johnson TW, Baby HM et al.* A novel small diameter nanotextile arterial graft is associated with surgical feasibility and safety and increased transmural endothelial ingrowth in pig. *J Nanobiotechnology*. 2022; 20: 71. doi: 10.1186/s12951-022-01268-1.
9. *Hisagi M, Nishimura T, Ono M, Gojo S, Nawata K, Kyo S.* New pre-clotting method for fibrin glue in a non-sealed graft used in an LVAD: the KYO method. *J Artif Organs.* 2010; 13: 174–177. doi: 10.1007/s10047-010-0504-1.
10. *Weadock KS, Goggins JA*. Vascular graft sealants. *J Long Term Eff Med Implants.* 1993; 3: 207–22.
11. *Copes F, Pien N, Van Vlierberghe S, Boccafoschi F, Mantovani D.* Collagen-Based Tissue Engineering Strategies

for Vascular Medicine. *Front Bioeng Biotechnol.* 2019; 7: 166. doi: 10.3389/fbioe.2019.00166.

12. *Zdrahala RJ.* Small caliber vascular grafts. Part I: state of the art. *J Biomater Appl.* 1996; 10: 309–329. doi: 10.1177/088532829601000402.
13. *Drury JK, Ashton TR, Cunningham JD, Maini R, Pollock JG.* Experimental and clinical experience with a gelatin impregnated Dacron prosthesis. *Ann Vasc Surg.* 1987; 1: 542–547.
14. *Fortin W, Bouchet M, Therasse E, Maire M, Héon H, Ajji A et al.* Negative *In Vivo* Results Despite Promising *In vitro* Data With a Coated Compliant Electrospun Polyurethane Vascular Graft. *J Surg Res.* 2022; 279: 491–504. doi: 10.1016/j.jss.2022.05.032.
15. *Huang F, Sun L, Zheng J. In vitro* and *in vivo* characterization of a silk fibroin-coated polyester vascular prosthesis. *Artif Organs.* 2008; 12: 932–941. doi: 10.1111/j.1525-1594.2008.00655.x.
16. *Lee JH, Kim WG, Kim SS, Lee JH, Lee HB.* Development and characterization of an alginate-impregnated polyester vascular graft. *J Biomed Mater Res.* 1997; 36: 200–208. doi: 10.1002/(sici)1097-4636(199708)36:2<200::aid-jbm8>3.0.co;2-o.
17. *Lisman A, Butruk B, Wasiak I, Ciach T.* Dextran/Albumin hydrogel sealant for Dacron(R) vascular prosthesis. *J Biomater Appl.* 2014; 28: 1386–1396. doi: 10.1177/0885328213509676.
18. *Madhavan K, Elliott WH, Bonani W, Monnet E, Tan W.* Mechanical and biocompatible characterizations of a readily available multilayer vascular graft. *J Biomed Mater Res B Appl Biomater.* 2013; 101: 506–519. doi: 10.1002/jbm.b.32851.
19. *Nemets EA, Pankina AP, Surguchenko VA, Sevastianov VI.* Bistability and cytotoxicity of medical devices based on cross-linked biopolymers. *Rus J Transpl Artif Organs.* 2018; 20 (1): 79–85. doi: 10.15825/1995-1191-2018-1-79-85.
20. *Glushkova TV, Ovcharenko EA, Rogulina NV, Klyshnikov KYu, Kudryavtseva YuA, Barbarash LS.* Dysfunction Patterns of Epoxy-Treated Tissue Heart Valves. *Cardiology.* 2019; 59 (10): 49–59. doi: 10.18087/cardio.2019.10.n327.
21. *Hennink WE, van Nostrum CF.* Novel crosslinking methods to design hydrogels. *Adv Drug Deliv Rev.* 2002; 54: 13–36. doi: 10.1016/s0169-409x(01)00240-x.
22. *Chernonosova VS, Laktionov PP.* Structural Aspects of Electrospun Scaffolds Intended for Prosthetics of Blood Vessels. *Polymers (Basel).* 2022; 14: 1698. doi: 10.3390/polym14091698.
23. *Fioretta ES, Simonet M, Smits AI, Baaijens FP, Bouten CV.* Differential response of endothelial and endothelial colony forming cells on electrospun scaffolds with distinct microfiber diameters. *Biomacromolecules.* 2014; 15: 821–829. doi: 10.1021/bm4016418.
24. *Azimi B, Nourpanah P, Rabiee M, Arbab SJ.* Poly (ε-caprolactone) Fiber: An Overview. *Engineered Fibers Fabrics.* 2014; 9: 74–90. doi: 10.1177/155892501400900309.
25. *Reid JA, McDonald A, Callanan A.* Electrospun fibre diameter and its effects on vascular smooth muscle cells. *J Mater Sci Mater Med.* 2021; 32: 131. doi: 10.1007/s10856-021-06605-8.
26. *Nemets EA, Surguchenko VA, Belov VYu, Xajrullina AI, Sevastyanov VI.* Porous Tubular Scaffolds for Tissue Engineering Structures of Small Diameter Blood Vessels. *Inorganic Materials: Applied Research.* 2023; 14: 400–407. doi: 10.1134/S2075113323020338.
27. *Lebedev AV, Bojko AI.* Zavisimost' prochnosti svarennykh krovenosnykh sosudov ot diametra, tolshchiny i modulya Yunga stenki. *Biomedical Engineering and Electronics.* 2014; 2: 54–61.

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EQUIVALENTS OF THE NEUTROPHIL-TO-LYMPHOCYTE RATIO OF CIRCULATING POOL OF STEM AND IMMATURE HEMATOPOIETIC CELLS FOR ASSESSING LIVER TRANSPLANT STATUS

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Objective: to study the applicability of the neutrophil-to-lymphocyte ratio (NLR) for monitoring recipient status and for possible minimization of maintenance immunosuppression in the long-term period after liver transplantation (LT). **Materials and methods.** Blood samples of 19 recipients with satisfactory graft function were examined by flow cytofluorometry at various time periods after LT using hematopoietic stem cell markers CD133, their CD31 derivatives, and alpha-fetoprotein (AFP), compared with the conventional NLR. **Results.** The use of NLR equivalents with CD133 and CD31 to assess liver transplant status is due to their high representation in liver tissue. Their values change in the long-term posttransplant period (from 1.5 to 6–7 years following LT) ≈20-fold and in different directions, but only when measuring their commissural to the liver cell fractions bearing the AFP marker. **Conclusion.** In contrast to the conventional NLR, maintenance of the lowest level of CD31 AFP, an NLR "equivalent", achieved at 1.5 years after LT, can be considered a criterion for the success of immunosuppressive therapy in the long-term post-LT period. The developed technique can be used to decide on whether to reduce or discontinue medication-assisted prophylaxis of graft rejection.

Keywords: neutrophil-to-lymphocyte ratio, hematopoietic stem cells (HSCs), CD133, CD31, alpha-fetoprotein, liver transplantation.

INTRODUCTION

The impact of immunosuppressive therapy in the long-term period after liver transplantation (LT) is associated with a whole set of complications that reduce the lifespan of recipients. Among the causes of negative outcomes, the leading ones are malignancy, infections, cardiovascular and nephrological problems [1]. Therefore, it is pertinent to search for rational ways to reduce the undesirable effects of post-LT immunosuppression. Based on analysis of modern literature, minimizing immunosuppression up to complete cancellation is considered the main approach, along with intraoperative and delayed tolerance induction, individualization and rationalization of regimens in order to reduce the incidence of side effects of drugs. From the clinical perspective, immune tolerance is defined as the preservation of stable graft function in a recipient who is not taking immunosuppressants. Unfortunately, the results of experimental studies on the mechanisms of tolerance have not yet revealed reliable biomarkers of tolerance [2]. Given the complexity and inconsistency of molecular mechanisms, the only reliable way to confirm tolerance is the absence of graft rejection after deliberate cessation of immunosuppression.

In the search for a reliable control of the recipient's condition while minimizing immunosuppression, the authors paid attention to the neutrophil-to-lymphocyte ratio (NLR), which is considered a simple and universal criterion for the severity of various human pathologic conditions [3]. Increased neutrophil count is a marker of inflammation, while low lymphocytes reflect stress and hypocellularity of hematopoietic tissue [4]. NLR can be used in the selection of prospective transplant patients [5]. NLR measured at 12 months after LT predicts overall survival over the next 7–9 years and correlates closely with markers of nutritional adequacy [6]. The shortening of lymphocyte telomeres with age exceeds that of granulocytes, indicating indirectly a greater expenditure of young lymphoid cells to ensure the vital activity of the body, and, possibly, the contribution of poorly differentiated lymphoid cells in the formation of prognostic properties of the NLR indicator. In addition, some young cells are "committed" to the liver tissue by the presence of the alpha-fetoprotein (AFP) marker [7–11].

The problem of long-term survival, as well as maintenance of the functional state of a liver transplant in some recipients, may be associated with depletion of the proliferative potential of the bone marrow lympho-

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cytic sprout (the product of the number of blood stem/progenitor lymphocytes by mitotic activity), the value of which limits life expectancy during natural aging of the body [12]. However, there is no direct information in the literature about the use of the indicator to control the volume of immunosuppressive therapy in the long-term period after LT. Taking into account all the features of immature cells, the authors suggested that the NLR equivalents with such cells can be a more sensitive indicator compared to the generally accepted NLR, in particular, in solving the problems of minimizing immunosuppressive therapy in the long term.

Objective: a comparative study of conventional NLR and its equivalents with blood cells of low differentiation/maturity for more reliable monitoring of recipients' condition and making further decisions on minimizing maintenance immunosuppressive therapy in the late post-LT period.

MATERIALS AND METHODS

Patients. The results of examination of 19 liver transplant recipients in the laboratory of transplantation and stem cell research at Granov Russian Research Center of Radiology and Surgical Technologies were studied. The patients were observed from 5 days to 120 months after LT, 9 men, 10 women. The average age during the operation was 44.9 ± 9.1 years. During the entire follow-up period, clinical and biochemical blood tests, abdominal ultrasound with elastometry were performed, tacrolimus blood levels were monitored and maintained at 3–5 ng/mL. Mean NLR at 1, 3, 5 and 10 years after LT was calculated. The distribution by nosologic variants before LT is shown in Fig. 1. Graft function was considered satisfactory if there were no deviations from normal serum levels of bilirubin, alanine aminotransferase (ALT) and aspartate aminotransferase (AST), alkaline phosphatase, and gamma-glutamyl transferase (GGT), if there were no circulatory disorders in the graft according to ultrasound and multi-slice spiral computed tomography (MSCT) data over time.

Materials. 7–8 mL blood samples were obtained at various times after LT and analyzed on the day of receipt, without storage. The viability of mononuclear cells (MNCs) from the entire interphase zone of the Ficoll-Paque density gradient was monitored by trypan blue exclusion test. Before cytometric phenotyping, cells were stained according to standard procedures to detect shapes in the synthetic (S) and mitotic (M) phases of the cell cycle with Hoechst 33342 reagent (bis(benzimidazole) fluorochrome; Sigma-Aldrich, St. Louis, Missouri, USA). CD133, CD31 cells, CD133AFP and CD31AFP double positive cells were stained using the standard Miltenyi Biotec protocol for CD133/2 antibodies conjugated to allophycocyanin (APC), BD Bioscience Pharmingen protocol for CD31 antibodies conjugated with fluorescein isothiocyanate (FITC), and R&D Systems protocol

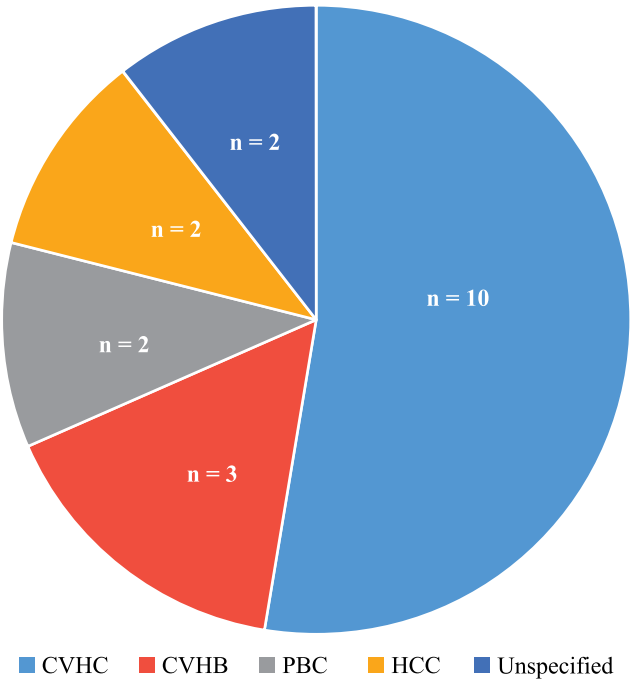

Fig. 1. Variants of liver cirrhosis before LT: CVHC, chronic viral hepatitis C; CVHB, chronic viral hepatitis B; PBC, primary biliary cholangitis; HCC, hepatocellular carcinoma; Unspecified, unspecified cirrhosis

for AFP antibodies conjugated with phycoerythrin (PE). An LSRFortessa flow cytometer (Becton-Dickinson, San Jose, CA, USA) was used.

Lymphocyte and granulocyte fractions were separated using forward scatter (FSC) and side scatter (SSC) light scatter plots, and cellular debris was excluded. Red laser (640 nm, 40 MW) was used to detect CD133+ cells, blue laser (480 nm, 50 MW) was used to detect AFP and CD31 cells, and ultraviolet (UV) laser (355 nm, 20 MW) for Hoechst 33342-labeled cells. The percentage of positive cells was calculated by subtracting the value for antibodies of the corresponding control isotype. At least 500,000 events were recorded twice to detect CD133 cells. A dot plot of Hoechst 33342 emission in blue (x-axis) and red (y-axis) wavelengths was used to separate the (G0 + G1), S and (G2 + M) phase events. Individual parameters were evaluated statistically with calculation of the mean value M, standard deviation (±σ) and standard error (±*m*). Mean values M were compared using the Student's t test and probability *p*. Parameter relationships in the graphs were analyzed by approximating the data points with regression lines that are automatically performed and described by mathematical functions in Excel, including the fit coefficient R^2.

The graphs show only those regression lines that had the maximum R^2 value, which means the marginal correspondence between the location of the points and the selected type of approximating curve / mathematical function from all those offered by the program (linear, exponential, exponential, power, logarithmic, step, po-

lynomial, parabolic). The equations for the regression lines are shown in the graphs. As a statistical measure of compliance of regression lines with the data entered into the program, we used the generally accepted criterion of reliability p (≤0.05), determined by t-test = $R/m_R = \sqrt{R^2 \times (n-2) / (1-R^2)}$ [12]. Equations for the regression lines are shown in the graphs.

RESULTS

The calculated mean NLR in recipients over a period of 3–5–10 years did not differ significantly – 2.19 ± 0.63, 2.17 ± 0.87 and 2.1 ± 0.58 according to the clinical blood test. The dynamics of neutrophil and lymphocyte content, according to flow cytometry data (see Figs. 2 and 3), is multidirectional, indicating maximum mean values of NLR up to 7 during the first 10 days after LT, followed by normalization to ≈1 by 250–500 days, and then repeated prolonged increase in NLR up to ≈4 by eight years after LT. Therefore, the entire study time was divided into relatively early and late periods. The primary decrease in NLR in the relatively early period, up to one and a half years, should be regarded as a positive effect of LT. It starts at a mean value slightly higher than the mean NLR level in those awaiting transplantation (see Fig. 3, black square in the graph), which has been determined in earlier studies. However, deviations of the mean NLR values in the early and, especially, in the late periods, were comparable to the data already known from literature, therefore, did not satisfy the objectives of the study.

Over time after LT, the percentage of lymphocytes in the synthetic phase (S phase) of the cell cycle increases, and the mitosis-to-synthesis (M/S) ratio decreases (see Fig. 4). The mean S phase in the late period (2.98 ± 0.74) is 8 times (p = 0.003) higher than that of the early period (0.35 ± 0.22). In contrast, the mean M/S ratio in the late period (0.051 ± 0.023) is 80 times (p = 0.003) smaller than the mean M/S ratio in the early period (4.25 ± 3.49), which, however, is not statistically confirmed (p = 0.21). This combination indicates a turbulent proliferation regime, classified as abnormal (synchronous). Nevertheless, the general downward trend in M/S in the combined periods (dashed line in Fig. 4) is confirmed by power law approximation: $M/S = 5.13x^{-0.649}$, $R^2 = 0.362$, $p < 0.001$. Thus, lymphocyte DNA synthesis in the long-term post-LT period increases, but this is not accompanied by increased mitotic activity, indicating turbulent lymphocytopoiesis and increased likelihood of cell apoptosis in the pre-mitotic phase of the cell cycle. In general, synthetic activity does not satisfy the task of the study, although it complements the characterization of the long-term period by a significant deficit of cell divisions in it.

The mean values of NLR equivalents in relatively early and late periods for CD133, CD133AFP, CD31, and CD31AFP subpopulations are presented in Table. Only the values of granulocyte pools (G) are shown, because the NLR is the result of arithmetic division of the percentage of the granulocyte subpopulation by the percentage of the lymphocyte subpopulation. The granulocytic constituents of NLR and its equivalents decrease by no more than 5-fold in the long-term period, except for CD133AFP. The expected decrease in the equiva-

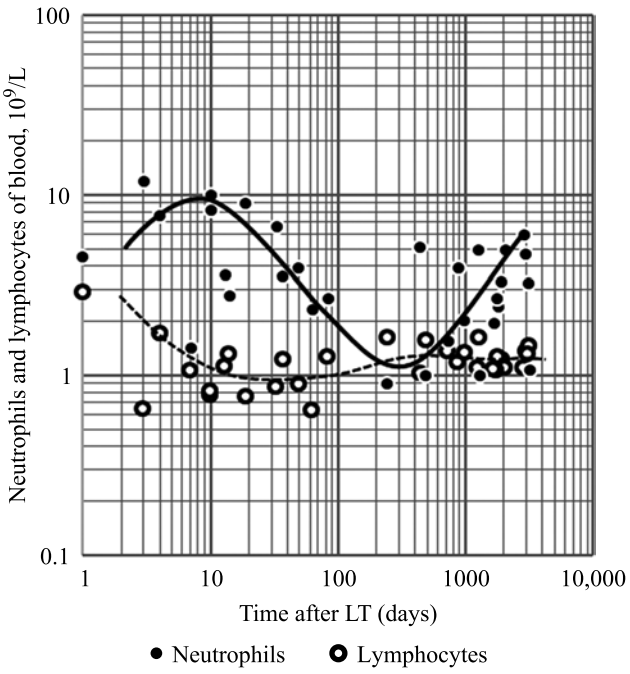

Fig. 2. Dynamics of lymphocytes and neutrophils over a long period after LT

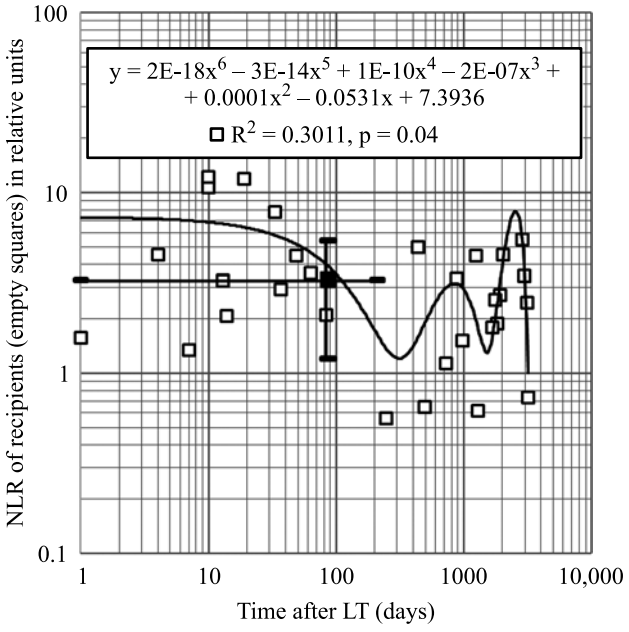

Fig. 3. Dynamics of normal NLR within the early (up to 350 days) and late (up to 3200 days) periods after LT. The approximation line shows the likely dynamics of the average indicator (p = 0.04). Black square: mean (M ± σ) for NLR of 12 patients from the LT waiting list

lents themselves does not occur, except for CD31AFP, where it is disproportionately large (≈20-fold), indicating a parity increase in the lymphocytic component of this equivalent as well. Based on period-averaged data (see Table) and comparison with conventional NLR, the CD31AFP subpopulation was not only quantitatively but also statistically preferable.

Consideration of the kinetic characteristics of CD31AFP confirms and complements this conclusion (see Fig. 5). The early decrease in NLR equivalent for liver-committed CD31AFP cells is significant (Fig. 5, center, Table), in contrast to non-committed CD31 (Fig. 5, left). The decrease in the values of the CD31AFP equivalent in the early period occurs in phase with a decrease in the conventional NLR indicator (Fig. 3), which can be interpreted as a favorable sign. There is a subsequent rise of CD31AFP in the long-term period due to a parallel rise in the granulocytic and a fall in the lymphocytic components of the equivalent (Fig. 5, right). This phenomenon may underlie late problems in recipients associated with maintenance immunosuppressive therapy. The kinetics of changes in the CD133AFP

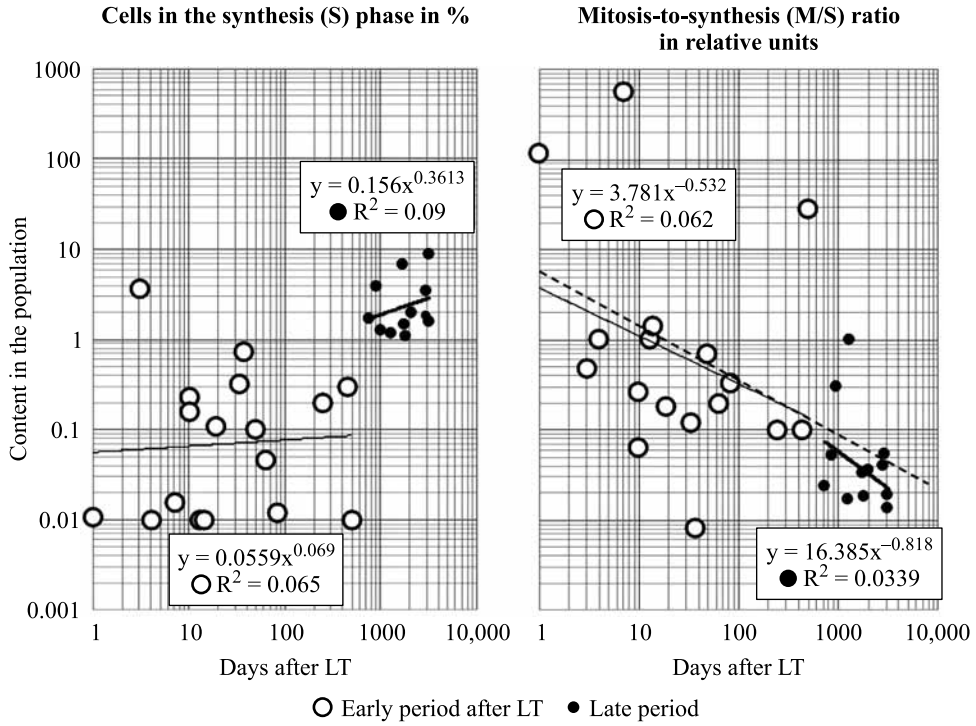

Fig. 4. Proliferative activity of lymphocytes after LT. The dotted line is the direction of change common to the two periods. P values for all equations >>0.05

Table

Mean values of "equivalents" of granulocyte/lymphocyte ratios (M ± m) for CD133, CD133AFP, CD31, CD31AFP subpopulations at relatively early (E) and late (L) periods after LT

Parameters	Normal NLR*	NLR equivalent				Mean time of early and late periods (M ± σ) in days
		CD 133	CD133AFP	CD31	CD31AFP	
Granulocytes, %	5.27 ± 0.83*	0.72 ± 0.145	0.308 ± 0.096	59.04 ± 6.36	57.33 ± 6.44	E 89 ± 153* E 29.7 ± 30
Granulocytes, %	3.1 ± 0.42*	0.257 ± 0.043	0.527 ± 0.428	12.83 ± 2.01	10.72 ± 2.1	L 1891 ± 850* L 1937 ± 905
p (between periods)	0.048*	0.008	0.62	<0.001	<0.001	<0.001* <0.001
NLR	6.8 ± 1.83*	13.85 ± 3.78	48.9 ± 22.57	10.9 ± 4.97	229.86 ± 60.65	E 89 ± 153* E 29.7 ± 30
NLR	2.6 ± 0.39*	7.97 ± 2.57	44.81 ± 11.74	2.89 ± 0.79	10.58 ± 3.44	L 1891 ± 850* L 1937 ± 905
p (between periods)	0.04*	0.21	0.53	0.13	0.003	<0.001* <0.001

* Data with normal NLR are given for comparison.

equivalent (Fig. 6, right) are inverted with respect to the CD31AFP equivalent. Its significant decrease in the long-term period (p = 0.0015) makes the CD133AFP equivalent the second contender for determining the graft condition, but only in the later stages, as its average values in two periods do not differ significantly (see Table). The decrease in CD133AFP in the long-term period is down to a significant decrease in the granulocytic component in the total CD133AFP pool and a moderate decrease in the lymphocytic component. The most probable mechanism for the inversion of kinetic trends of CD31AFP lymphocytes and CD31AFP granulocytes in the long-term period (Fig. 5, right) is that CD31 cells are the closest progeny of CD133 hematopoietic stem cells (HSC) in the series of sequential differentiation. In this case, quantitative changes in the opposite direction occur only when cells are produced in the mode of symmetric (depleted) hematopoiesis, which is confirmed by the growing deficit of mitotic activity (see Fig. 4).

So, we have identified two NLR equivalents – with CD133AFP and CD31AFP, which are promising for monitoring recipients in the long term, which significantly

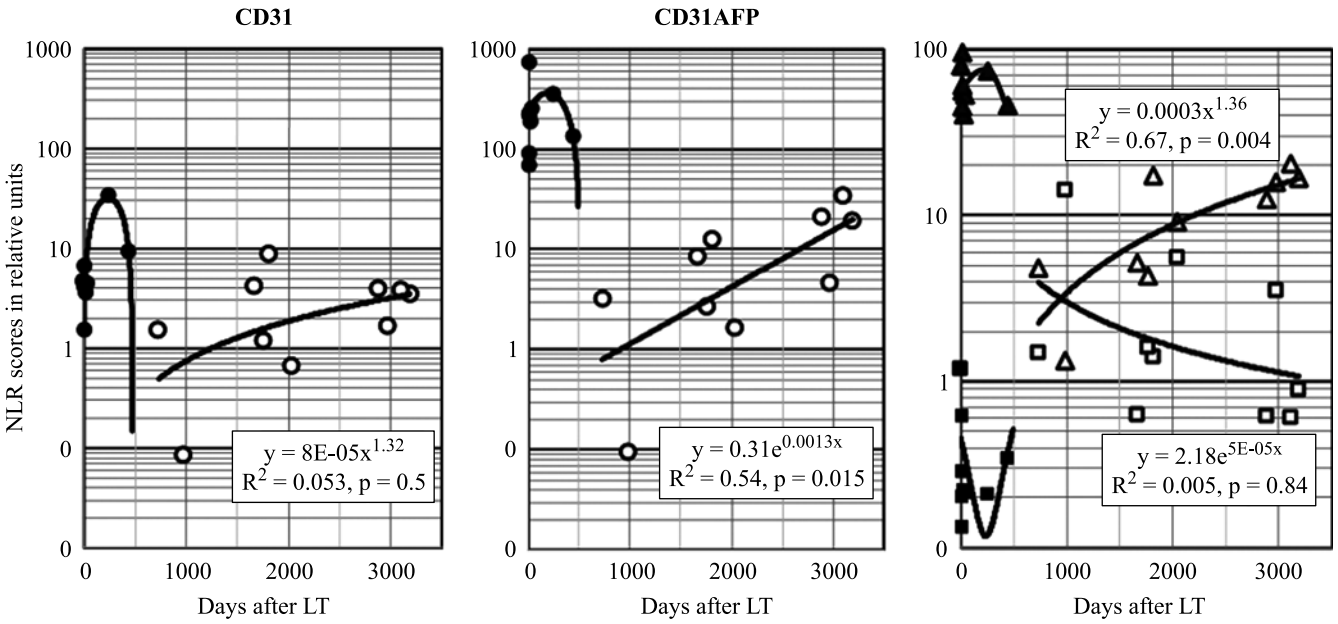

Fig. 5. Changes in NLR scores over time after LT for CD31 and CD31AFP subpopulations. Black icons represent the early period, white icons represent the late period. Solid approximation lines in Excel are given for both periods. Equations for the approximation lines are given in the boxes on the graphs for the late period only. Circles, NLR; triangles, CD31AFP in the granulocyte pool in %; squares, CD31AFP in the lymphocyte pool in %

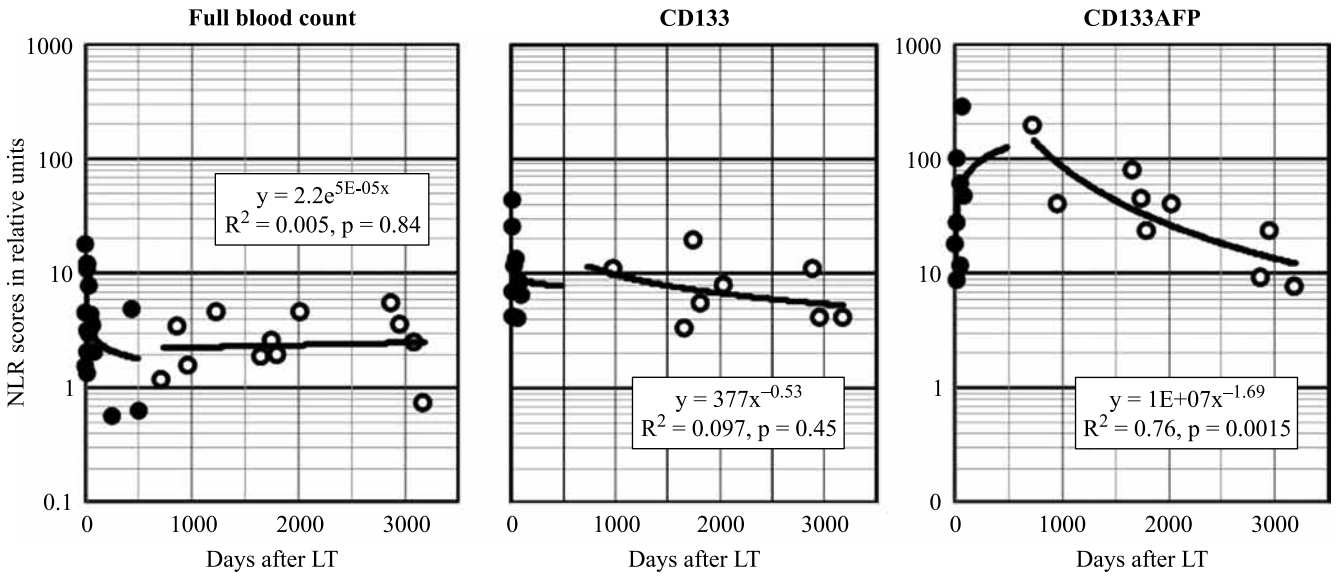

Fig. 6. Changes in NLR scores over time after LT for CD133 and CD133AFP subpopulations. Black icons represent the early period, white icons represent the late period. Equations for the approximation lines are given in the boxes on the graph

exceed the capabilities of conventional NLR. Both equivalents change smoothly and statistically significantly over the long-term period ≈20-fold, whereas the conventional NLR is virtually unchanged (Fig. 3, dashed line; Fig. 6, left) between 17 and 106 months after LT. Oppositely-directed exponential changes in the two NLR equivalents occur with a doubling period of ≈1.5 years. The prognostic capabilities of these indicators require further studies in the context of immunosuppressive therapy minimization.

DISCUSSION

Based on data obtained, the relatively early period from 0 to 1.5 years after LT seems to be optimal for identifying recipients that are most resistant to graft rejection according to the criterion of the maximum rate of decrease in the CD31AFP equivalent value. Over the late period, the NLR equivalent CD31AFP steadily increased. In parallel, there was a slow depletion of poorly differentiated, morphogenic, CD31AFP-lymphocytes committed to the liver tissue. At the same time, the opposite dynamics of lymphoid and myeloid components of CD31AFP cells may reflect the gradual depletion of lymphopoiesis with the predominance of the myeloid component over the depleting lymphoid one, like what happens in natural aging [13].

A significant reduction in the total pool of CD133AFP stem cells among 133AFP granulocytes ($p < 0.001$), along with a moderate decrease in 133AFP lymphocytes ($p = 0.06$), form a late decrease in NLR equivalent CD133AFP. Since in normal liver, there should be a constant repopulation of either pluripotent or rapidly dividing young AFP-positive cells, the identified changes may signify a progressively increasing threat to graft viability and recipient [14]. In the long-term post-LT period, one can assume the development of devascularization processes with the subsequent development of fibrosis, which requires further study.

If we allow for the influence of cells from the circulation on the migrant spectrum directly in the transplanted liver tissue, it first normalizes to optimal by 1–1.5 years after LT and then gradually depletes by 8–9 years.

Data from modern studies have proven the morphogenic properties of HSCs and their immediate undifferentiated progeny. For instance, the comparative analysis of marker composition in a normal liver showed that, like placental tissue, it is strongly polarized towards the predominance of young migrant cells compared to their content in blood [15], HSCs of double positivity for CD34 and CD133 give rise to both early endothelial precursors CD31 [16] and lymphoid stem cell lineage with terminal deoxynucleotidyl transferase marker TdT+ [15, 17–19].

Programmed death ligand-1 (PD-L1, CD274) plays an important role in processes such as tissue transplantation, pregnancy, autoimmune diseases, hepatitis, etc. [20]. Its expression on circulating CD34 HSCs closely correlates with T cell apoptosis. Apoptosis is associated with subsequent delivery of TdT into the intercellular medium and reutilization of degradation products by neighboring viable cells during regeneration [7].

Terminal-interacting protein deoxynucleotidyl transferase enhances the proliferative activity of TdT+ and vasculogenic properties of CD34 HSC [20]. Therefore, according to the authors' opinion, NLR equivalents have more informative value when evaluating recipient and graft condition in the long-term post-LT period, as well as when trying to minimize maintenance immunosuppressive therapy as a monitoring component.

CONCLUSION

Results obtained from the study suggest that maintenance of the lowest level of NLR equivalent CD31AFP, achieved by 1.5 years after LT, can be considered a criterion for the adequacy of maintenance immunosuppressive therapy in the longer period. The developed method for monitoring recipient and liver graft conditions can be used for decision making and monitoring when reducing or stopping immunosuppression.

The authors declare no conflict of interest.

REFERENCES

1. *Aberg F, Gissler M, Karlsen TH, Ericzon BG, Foss A, Rasmussen A et al.* Differences in long-term survival among liver transplant recipients and the general population: a population-based Nordic study. *Hepatology*. 2015; 61 (2): 668–677. doi: 10.1002/hep.27538.
2. *Shevchenko OP, Kurabekova RM, Tsiroulnikova OM*. Biomarkers of immune tolerance in liver transplantation. *Russian Journal of Transplantology and Artificial Organs*. 2016; 18 (3): 137–144. doi.org/10.15825/1995-1191-2016-3-137-144.
3. *Lunkov VD, Maevskaya MV, Tsvetaeva EK, Mendez AG, Zharkova MS, Tkachenko PE, Ivashkin VT.* Neutrophil to Lymphocyte Ratio as a Predictor of Adverse Outcome in Patients with Decompensated Liver Cirrhosis. *Russian Journal of Gastroenterology, Hepatology, Coloproctology*. 2019; 29 (1): 47–61. https://doi.org/10.22416/1382-4376-2019-29-1-47-61.
4. *Fock RA, Blatt SL Beutler B, Pereira J, Tsujita M, de Barros FEV, Borelli P.* Study of lymphocyte subpopulations in bone marrow in a model of protein-energy malnutrition. *Nutrition*. 2010; 26: 1021–1028. doi: 10.1016/j.nut.2009.08.026.
5. *Angelico R, Parente A, Manzia TM.* Using a weaning immunosuppression protocol in liver transplantation recipients with hepatocellular carcinoma: a compromise between the risk of recurrence and the risk of rejection? *Transl Gastroenterol Hepatol*. 2017; 21 (2): 74. doi: 10.21037/tgh.2017.08.07. PMID: 29034347.
6. *Pravisani R, Mocchegiani F, Isola M, Lorenzin D, Adani GL, Cherchi V et al.* Postoperative trends and prognostic values of inflammatory and nutritional biomarkers

after liver transplantation for hepatocellular carcinoma. *Cancers (Basel)*. 2021; 13 (3): 513. doi: 10.3390/cancers13030513. PMID: 33572776.
7. *Lansdorp PM*. Telomeres stem cells, and hematology. *Blood*. 2008; 111 (4): 1759–1766. doi: 10.1182/blood-2007-09-084913.
8. *Lin BY, Zhou L, Geng L, Zheng ZY, Jia JJ, Zhang J et al.* High neutrophil-lymphocyte ratio indicates poor prognosis for acute-on-chronic liver failure after liver transplantation. *World J Gastroenterol*. 2015; 21 (11): 3317–3324. doi: 10.3748/wjg.v21.i11.3317. PMID: 25805939.
9. *Shoutko AN.* The possible involvement of apoptotic decay of terminal deoxynucleotidyl transferase-positive lymphocytes in the reutilization of the extracellular DNA fragments by surrounding living cells. *Open J of Biophysics*. 2021; 11 (04): 371–382. doi: 10.4236/ojbiphy.2021.114014.
10. *Huang L, Zheng Y, Yuan X, Ma Y, Xie G, Wang W et al.* Decreased frequencies and impaired functions of the $CD31^+$ subpopulation in T_{reg} cells associated with decreased FoxP3 expression and enhanced T_{reg} cell defects in patients with coronary heart disease. *Clin Exp Immunol.* 2017; 187 (3): 441–454. doi: 10.1111/cei.12897. PMID: 27997991.
11. *Shoutko AN, Gerasimova OA, Marchenko NV, Zherebtsov FK*. Induction of circulating CD133+ stem cells committed to cirrhotic livers in waitlisted patients. *Russian Journal of Transplantology and Artificial Organs*. 2020; 22 (4): 43–51. https://doi.org/10.15825/1995-1191-2020-4-43-51.
12. *Shoutko AN, Gerasimova OA, Ekimova LP, Zherebtsov FK, Mus VF, Matyurin KS et al*. Lymphocyte reproductive activity normalized to numbers of hematopoietic stem cells in blood and rate of death in fatal diseases. *Int J of Genetics and Genomics*. 2017; 5: 54–62. doi: 10.11648/j.ijgg.20170505.12.
13. *Lué A, Solanas E, Baptista P, Lorente S, Araiz JJ, Garcia-Gil A et al.* How important is donor age in liver transplantation? World J Gastroenterol 2016 June 7; 22 (21): 4966–4976. doi: 10.3748/wjg.v22.i21.4966.
14. *Lombard CA, Prigent J, Sokal EM.* Human Liver Progenitor Cells for Liver Repair. *Cell Med*. 2013; 5 (1): 1–16. doi: 10.3727/215517913X666459.
15. *Shoutko AN.* Tissues Protein Microenvironment and Survival by Age at Cancers. *Acta Scientific Cancer Biology*. 2022; 6: 20–27. doi: 10.31080/ASCB.2022.06.0380.
16. *Drzewiecki K, Choi J, Brancale J, Leney-Greene MA, Sari S, Dalgiç B et al.* GIMAP5 maintains liver endothelial cell homeostasis and prevents portal hypertension. *J Exp Med.* 2021; 7 (218): e20201745. https://doi.org/10.1084/jem.20201745.
17. *Hu M, Li S, Menon S, Liu B, Hu MS, Longaker MT et al.* Expansion and hepatic differentiation of adult blood-derived CD34+ progenitor cells and promotion of liver regeneration after acute injury. *Stem Cells Transl Med*. 2016; 5 (6): 723–732. doi: 10.5966/sctm.2015-0268.
18. *Schwartzenberg S, Mor A, Luboshits G, Planer D, Deutsch V, Keren G et al.* Association between circulating early endothelial progenitors and CD4+CD25+ regulatory T cells: A possible cross-talk between immunity and angiogenesis? *American Journal of Immunology.* 2005; 1 (4): 143–147. doi: 10.3844/ajisp.2005.143.147.
19. *Billaud M, Donnenberg VS, Ellis BW, Meyer EM, Donnenberg AD, Hill JC et al.* Classification and functional characterization of vasa vasorum-associated perivascular progenitor cells in human aorta. *Stem Cell Reports*. 2017; 9 (1): 292–303. doi: 10.1016/j.stemcr.2017.04.028.
20. *Lee JU, Levit R, Yoon YS.* Human peripheral blood-derived CD31+ cells have robust angiogenic and vasculogenic properties and are effective for treating ischemic vascular disease. *J Am Coll Cardiol*. 2010; 56 (7): 593–607. doi: 10.1016/j.jacc.2010.01.070.
21. *Abdellatif H, Shiha G.* PD-L1 Expression on circulating CD34+ hematopoietic stem cells closely correlated with T-cell apoptosis in chronic hepatitis C infected patients. *Int J Stem Cells*. 2018; 11 (1): 78–86. doi: 10.15283/ijsc17047.

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DEVELOPMENT OF A NEW LOW-VOLUME OXYGENATOR AND CREATION OF A HYDRODYNAMIC TEST BENCH FOR *EX VIVO* LUNG PERFUSION IN SMALL ANIMALS

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Small animal models are widely used in basic research. However, experimental hydrodynamic test benches, which include extracorporeal circuits, often have limitations associated with the size and filling volume of equipment. Thus, we aimed at developing and validating a miniature oxygenator as well as a low-volume hydrodynamic system for *ex vivo* perfusion of small animal lungs. A series of low-volume membrane oxygenators (n = 10) with 90–100 aligned microporous polypropylene hollow fibers, placed inside a sheath that is sealed at both ends to isolate the perfusing solution, was designed and manufactured. This design makes gas to flow through the hollow fibers and perfusate to circulate around the fibers. A low-volume hydrodynamic test bench was designed and assembled for isolated *ex vivo* lung perfusion and for evaluation of the performance characteristics of the oxygenators: gas and perfusate flow, perfusion pressure and temperature at 5–70 ml/min flow range.

Keywords: low-volume membrane oxygenator, oxygenator, isolated perfused organ, lung perfusion, hydrodynamic test bench, blood oxygenation.

INTRODUCTION

The development of extracorporeal membrane oxygenators for cardiac surgery has reached a high level of quality and reliability [1–4]. Currently, there are several systems available for adult patients, children, and newborns. However, even the most miniaturized systems are not suitable for basic protocols for experimental studies in small animal models with extracorporeal blood circulation or for perfusion of their isolated organs. Reducing the volume of oxygenators used in such experimental studies is critical to the usability of the system, since the filling volume of the oxygenator usually accounts for the bulk of the total filling volume of the system.

Currently, some "homemade" low-fill oxygenators have been described in the literature (see Table 1). However, most studies still require a device with a primary fill volume of a few milliliters, which requires additional volumes of donated animal blood [5–10].

In addition to technical difficulties, miniaturization of the oxygenator is a challenge in terms of its functionality and efficiency to guarantee the reproducibility and accuracy of the experiment [11–13].

So, the development of a low-volume oxygenator will allow to develop of standard perfusion systems for experimental studies involving small animals or their isolated organs. In this paper, we present a description of our own development of a low-volume membrane oxygenator, its characteristics that prove the functionality and reliability of the oxygenator, as well as a new hydrodynamic test bench for perfusion of small animal lungs using the oxygenator of our own design.

Table 1

Brief properties and characteristics of previously developed low-volume oxygenators

Author	Fill volume (mL)	Perfusate flow rate (mL/min)	Oxygenator size (mm)	Perfusion time (min)
Kim W.G.	29	21.2	–	30
Gunzinger R.	4	54	40 × 40 × 15	60
Jungwirth B.	4	57–64	128 × 27	45–105
Ordodi V.L.	8	17–42	–	180
Dong G.H.	4	50–75	–	60
Shang H.W.	10	14–40	–	60

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MATERIALS AND METHODS

Design features for the development of a low-volume oxygenator

An ideal 3D design of a new mini-oxygenator with the following given parameters was designed: total length of the oxygenator, 10 cm; inner diameter, 5 mm; outer diameter, 7 mm; dry weight of the oxygenator, up to 15 g; average number of fibers, 90–95 units; total effective working surface area, up to 90 cm^2; perfusate flow rate, up to 80 mL/min. The block diagram of operation and 3D design of the low-volume oxygenator is presented in Fig. 1.

The physical model of the oxygenator is a housing consisting of two $^1/_4 \times {}^1/_4$ inch disposable polystyrene connectors, each with one Luer port (Maquet Cardiopulmonary AG, Hirlingen, Germany), connected by silicone tubing (Raumedic AG, Helmbrechts, Germany) and symmetrically oriented (see Fig. 2).

Microporous polypropylene hollow fibers (Oxyphan PP50/200, Membrana GmgH, Wuppertal, Germany) placed in our housing are sealed together with connectors at each end of the shell using epoxy resin (bisphenol A/F-based epoxy resin and modified cycloaliphatic amine hardener, Epoxy Master, Russia). This design isolates the

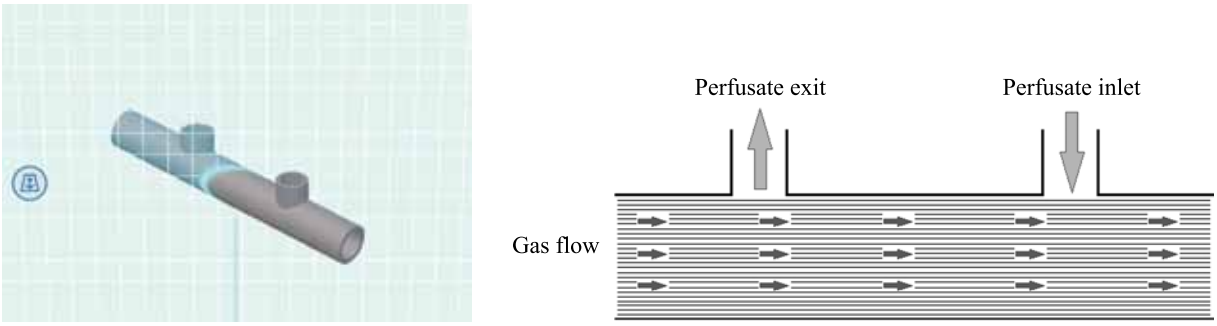

Fig. 1. On the left is an ideal model of the designed low-volume oxygenator. On the right is a block diagram of how the low-volume oxygenator operates

Fig. 2. A developed experimental model of the low-volume oxygenator

gas compartment, where gas passes through the interior of the hollow fibers, from the perfusate compartment, where the solution circulates around the hollow fibers from the outside. The perfusate flows through the low-volume oxygenator countercurrent to the gas flow.

A 3D cross-sectional model of the sealed ends of the oxygenator was designed to estimate the required effective working surface area of the fibers (see Fig. 3), and a cross-section of the designed oxygenator was taken to compare theoretical calculations and actual results obtained (see Fig. 4).

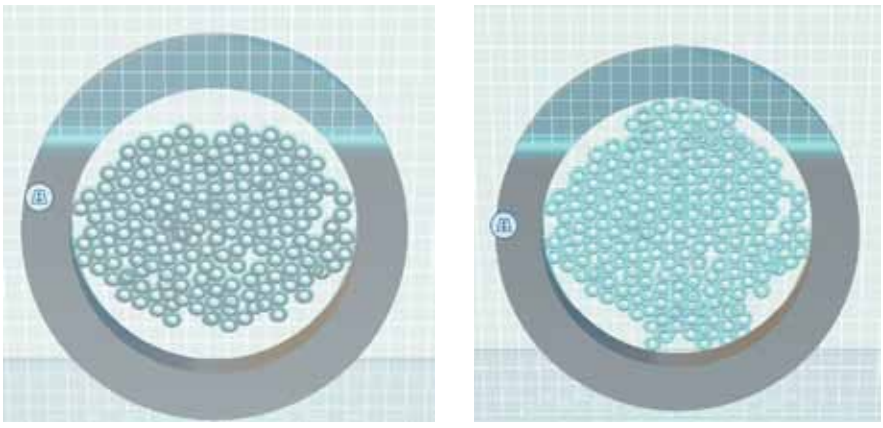

Fig. 3. A 3D model of the cross section of the ends of the oxygenator

The theoretical calculations were 98% consistent with the practical calculations (at 0.05% confidence interval with the general population parameter at the estimated confidence level), i.e., more than 95% of the polypropylene hollow fibers were open at both ends.

In vitro performance assessment of the developed low-volume oxygenators

The oxygenation capacity of the developed low-volume oxygenators was tested using modified Krebs–Henseleit (KH) buffer in vitro on a hydrodynamic perfusion recirculation bench. A Diagram of the test bench is shown in Fig. 5.

The KH buffer was freshly prepared for each experiment (mmol): NaCl 118, KCl 4.7, KH_2PO_4 1.2, $MgSO_4 \cdot 7H_2O$ 1.2, $CaCl_2 \cdot 2H_2O$ 1.25, $NaHCO_3$ 25 and glucose 11. The hydrodynamic test bench contained a deoxygenation tank in which the perfusate was bubbled with a 95% N_2 / 5% CO_2 gas mixture and a low-volume oxygenator through which 95% O_2 / 5% CO_2 was passed.

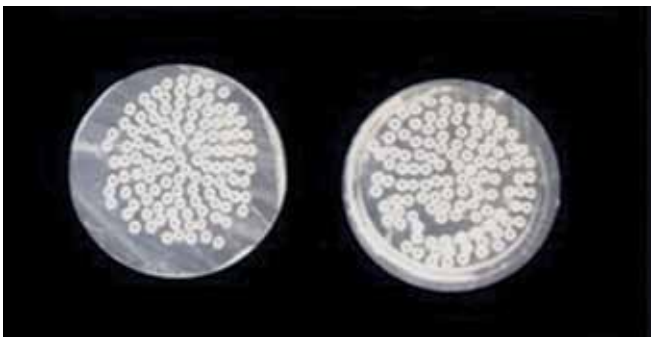

Fig. 4. Visualization of slices of the sealed ends of the oxygenator using a digital microscope

N_2/CO_2 was maintained at 0.4 L/min, and O_2/CO_2 at 0.8–1.2 L/min. A peristaltic pump was used to circulate perfusate from the tank through the oxygenator and back to the tank. Ports were also installed in the circuit to draw perfusate before and after passing through the low-volume oxygenator.

Perfusate pressure was recorded using pressure sensors (Edwards Lifesciences, USA). Flow sensors (Transonic Systems, USA) and temperature sensors were included in the circuit immediately before and after the oxygenator. Flow and pressure measurements were continuously recorded using an Angioton multichannel module (Biosoft-M, Russia) on a Pumpax high-performance data acquisition system (Biosoft-M, Russia). The partial pressure of oxygen (pO_2) of oxygenated and deoxygenated buffer, gas flow rate, pre- and post-oxygenator perfusate pressure, and temperature were measured every 10 minutes at perfusate flow rates ranging from 5 to 65 mL/min. For the duration of the experiment (90 minutes), the gas mixture was heated to 37.0 °C using a thermostat and water bath (XMTE-205, China).

Design and development of a new isolated ex *vivo* system for small animal lung perfusion using the new oxygenator

A schematic diagram of a hydrodynamic test bench for *ex vivo* perfusion of small animal lungs using a new low-volume oxygenator was developed (see Fig. 6).

An analytical review of the assembly technique, positive pressure ventilation settings, perfusate composition, flow rate conditions and lung cannulation, was carried out. Based on its results and on the block diagram, the hydrodynamic test bench, presented in Fig. 7, was assembled.

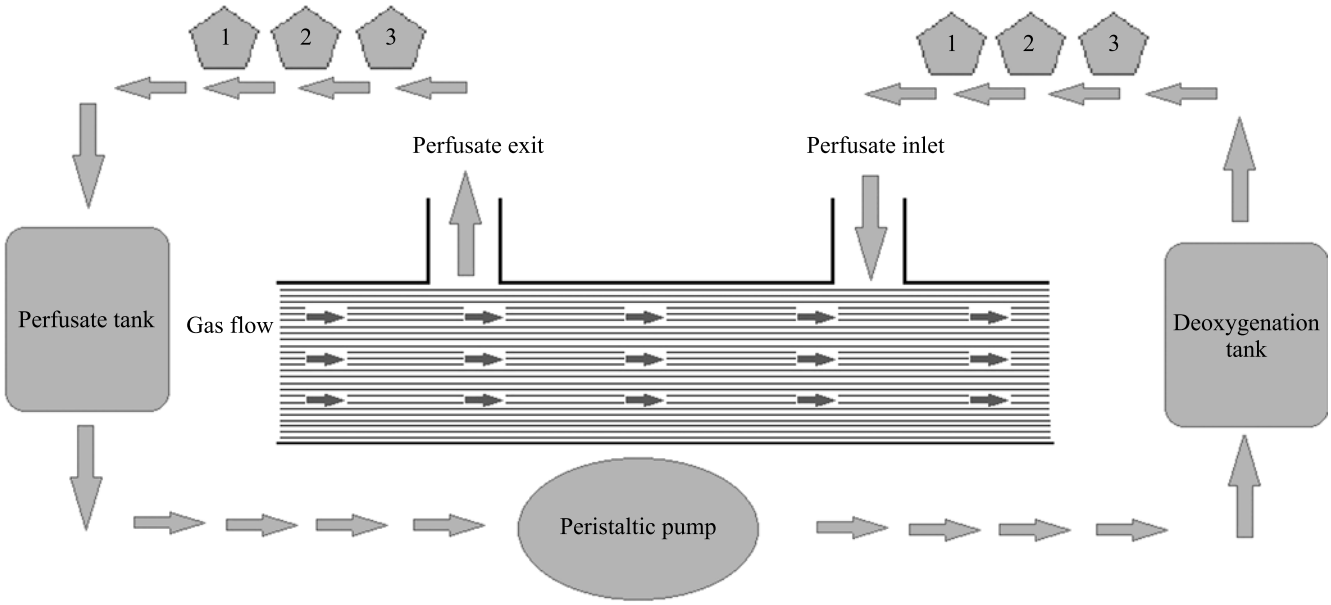

Fig. 5. Diagram of the hydrodynamic test bench for the study of the developed oxygenators in vitro (1, 2, 3 – temperature and pressure sensors and a sampling port)

Once all cannulas were inserted and connected to the circuit, we made sure that the lungs were ventilated and there were no perfusate leaks along the entire line. Oxygen exchange increased as soon as the ventilator was turned on and inflated the lungs to engage more alveoli for gas exchange. We have further research ahead of us to optimize the hydrodynamic test bench and further develop an *ex vivo* lung perfusion technique.

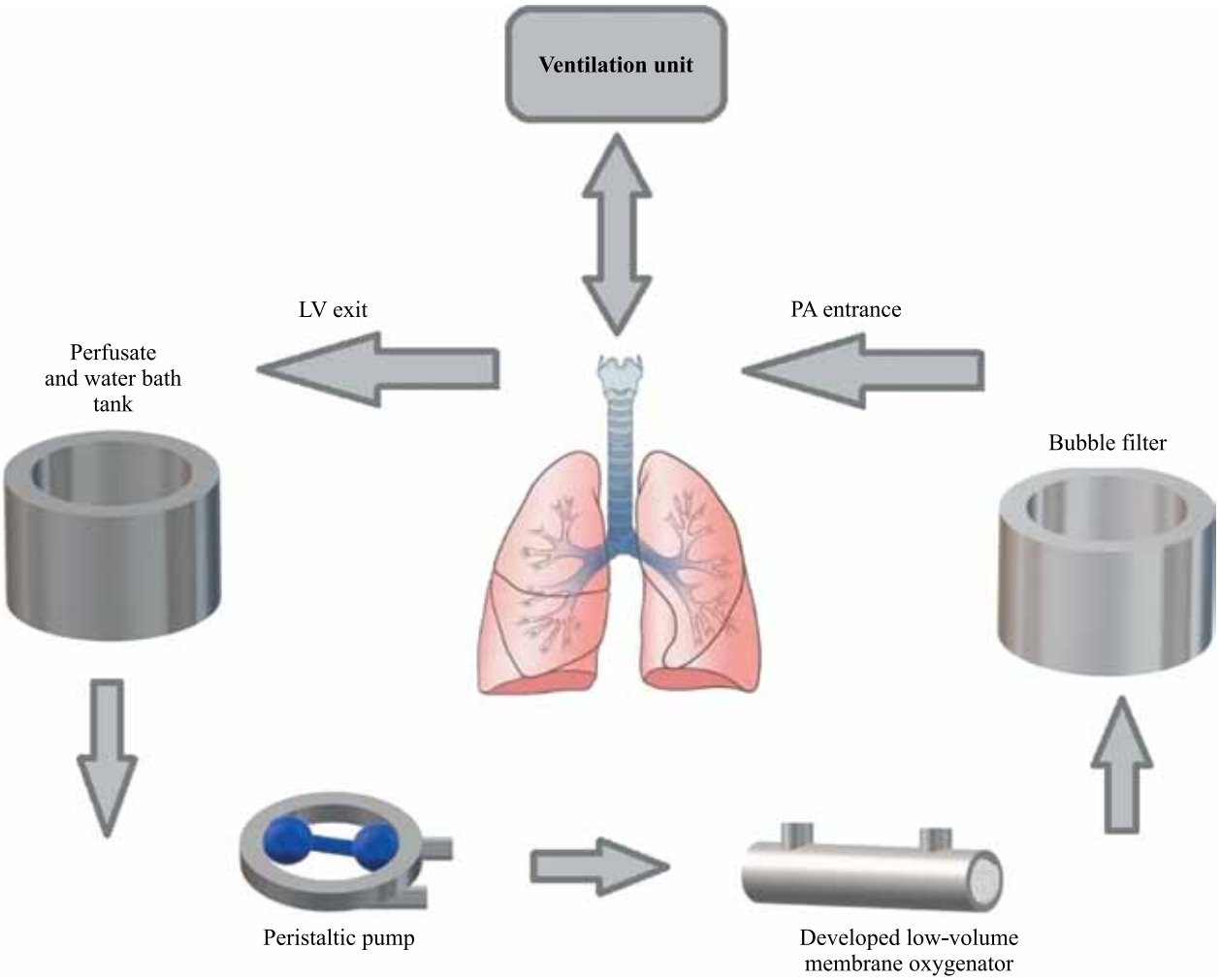

Fig. 6. Diagram of hydrodynamic test bench for isolated *ex vivo* perfusion of small animal lung

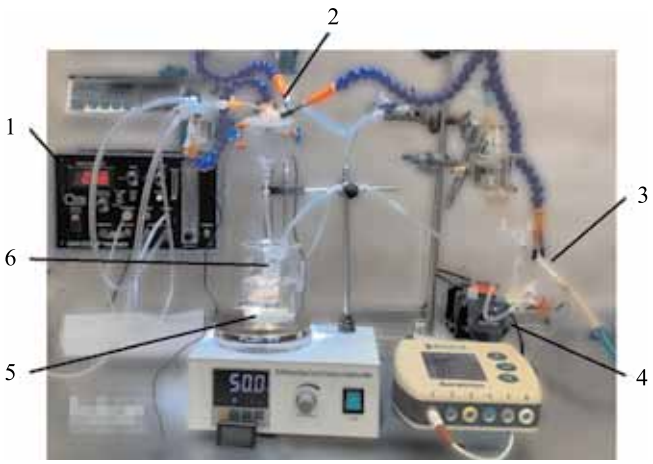

Fig. 7. Hydrodynamic test bench for isolated *ex vivo* perfusion of small animal lungs. 1, ventilation unit; 2, small animal donor organs; 3, new low-volume oxygenator; 4, peristaltic pump; 5, water bath; 6, perfusate tank

RESULTS

A series of low-volume membrane oxygenators (N = 10) was designed and developed. Table 2 summarizes the calculated and obtained physical characteristics of the oxygenators.

The average filling volume of the developed oxygenator is 1.5 ± 0.5 ml.

Experimental studies on the hydrodynamic test bench to evaluate the operation of an oxygenator with a KH buffer with a range of perfusate flow rate variation from 5 to 70 mL/min allowed us to obtain the following indicator values – pO_2 after passing through the oxygenator varied on average from 400 to 500 mmHg; the value averaged from 100 to 200 mmHg after the deoxygenation tank. Transport of oxygen and PO_2 in the oxygenated buffer gradually decreased with increasing perfusate flow rate;

Table 2

Theoretical and actual characteristics of low-volume membrane oxygenators

	Theoretical value	Actual value
Oxygenator total length (cm)	10	13
Oxygenator outer diameter (mm)	5	5–6
Oxygenator inner diameter (mm)	7	7–8
Oxygenator weight (g)	≤15	12.7 ± 1.1
Average number of open fibers (units)	90–95	100 ± 8
Effective working surface of fiber line (cm^2)	90	78 ± 6
Perfusate flow rate (mL/min)	≤ 80	≤70

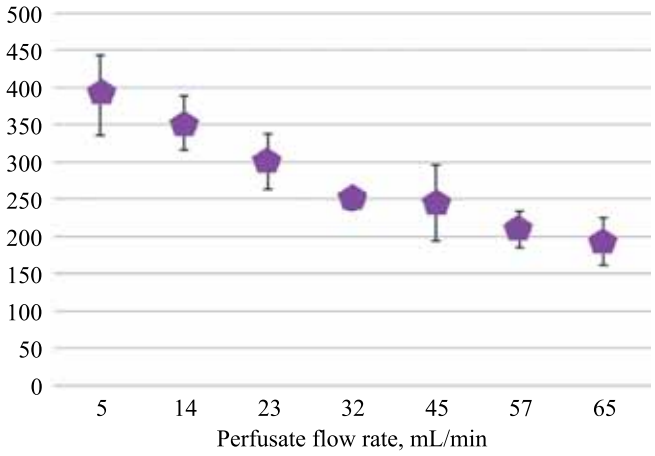

Fig. 8. Oxygen transport provided by the oxygenator

whereas PO_2 gradually increased in the deoxygenated buffer (see Fig. 8).

The developed hydrodynamic test bench for isolated *ex vivo* perfusion revealed no obvious problems. So, we plan to conduct a series of research studies with small animals.

DISCUSSION

We have developed an efficient mini-oxygenator with a very small filling volume of ≈1.2 mL, which is one of the lowest values currently available among membrane oxygenators. Our oxygenator efficiently oxygenates the KH buffer at perfusate flow rates of up to 70 mL/min, providing a pO_2 ≈400 mmHg for at least 90 minutes.

There is now a wealth of information in the public domain on small animal models (rats), which are of great value in terms of investigating many aspects of the cardiovascular and cardiopulmonary systems [11, 14–20]. Evaluation of inflammatory response, evaluation of solutions, activation of the coagulation system, biocompatibility of new materials – these and many other aspects of medical practice can be studied in experimental animal models. In these situations, a miniaturized hydrodynamic system is convenient and effective, especially if the experiment requires a blood-filled circuit. It is for such circuits with low perfusate filling volumes that an oxygenator such as the one that has been developed is required.

Although a series of several mini-oxygenators with filling volumes <2.0 mL were produced, some differences between the samples, influencing the initial evaluation of the developed mini-oxygenator, were observed. The results presented in this article should be considered as a starting step towards development of more advanced models and optimization of its manufacture.

The potential for these oxygenators is high. Of great research and practical interest are the data obtained when using the mini-oxygenator as part of a circuit on the developed hydrodynamic test bench for isolated *ex vivo* perfusion of lungs of small animals. Both the mini oxygenator and the miniature hydrodynamic bench open fundamentally new possibilities in the provision of technical support for experiments on *ex-vivo* perfusion of lungs on small laboratory animal models.

CONCLUSION

A mini-oxygenator has been developed and its functionality evaluated. The oxygenator is efficient and reliable for oxygenating physiological buffers over the range of flow rates commonly used in small laboratory animal models.

The performance parameters of the oxygenator remain stable for at least 90 minutes, which is a sufficient time for most experimental protocols.

The created oxygenator made it possible to develop experimental protocols that were impossible to implement due to perfusate volume and/or size of available oxygenators. For instance, the hydrodynamic test bench for isolated *ex vivo* perfusion of small animal lungs is relevant for the study and development of proprietary methods and approaches for *ex-vivo* perfusion of donor lungs. Further research in this area will be pursued.

The authors declare no conflict of interest.

REFERENCES

1. *Fu X, Su Z, Wang Y, Sun A, Wang L, Deng X et al.* Comparison of hemodynamic features and thrombosis risk of membrane oxygenators with different structures: A numerical study. *Comput Biol Med*. 2023; 159: 106907.
2. *De Vroege R, Wagemakers M, Te Velthuis H, Bulder E, Paulus R, Huybregts R et al.* Comparison of three commercially available hollow fiber oxygenators: Gas transfer performance and biocompatibility. *ASAIO J*. 2001; 47: 37–44.
3. *Berner M, Clement D, Stadelmann M, Kistler M, Boone Y, Carrel TP et al.* Development of an ultra mini-oxygenator for use in low-volume, bufferperfused preparations. *Int J Artif Organs*. 2012; 35: 308–315.
4. *Karimova A, Robertson A, Cross N, Smith L, O'Callaghan M, Tuleu C et al.* A wet-primed extracorporeal membrane oxygenation circuit with hollowfiber mem-

brane oxygenator maintains adequate function for use during cardiopulmonary resuscitation after 2 weeks on standby. *Crit Care Med*. 2005; 33: 1572–1576.
5. *Kim WG, Choi SH, Kim JH.* Temperature control using a heat exchanger of a cardioplegic system in cardiopulmonary bypass model for rats. *Artif Organs*. 2008; 32 (12): 993–998.
6. *Günzinger R, Wildhirt SM, Schad H, Heimisch W, Gurdan M, Mendler N et al.* A rat model of cardiopulmonary bypass with cardioplegic arrest and hemodynamic assessment by conductance catheter technique. *Basic Res Cardiol*. 2007; 102 (6): 508–517.
7. *Jungwirth B, Mackensen GB, Blobner M, Neff F, Reichart B, Kochs EF, Nollert G.* Neurologic outcome after cardiopulmonary bypass with deep hypothermic circulatory arrest in rats: description of a new model. *J Thorac Cardiovasc Surg*. 2006; 131 (4): 805–812.
8. *Ordodi VL, Paunescu V, Mic AA, Ionac M, Sandesc D, Mic FA.* A small scale oxygenator for cardiopulmonary bypass in rats. *Int J Artif Organs*. 2006; 29 (8): 750–755.
9. *Dong GH, Xu B, Wang CT, Qian JJ, Liu H, Huang G et al.* A rat model of cardiopulmonary bypass with excellent survival. *J Surg Res*. 2005; 123 (2): 171–175.
10. *Shang HW, Xiao YB, Liu M, Chen L.* Establishment of an animal model of non-transthoracic cardiopulmonary bypass in rats. *Chin J Traumatol*. 2005; 8 (5): 289–292.
11. *Alexander B, Von Arnem T, Aslam M, Kolhe PS, Benjamin IS.* Practical miniaturized membrane oxygenator for isolated organ perfusion. *Lab Invest*. 1984; 50 (5): 597–603.
12. *You XM, Nasrallah F, Darling E, Robins M, Nieman G, Searles B.* Rat cardiopulmonary bypass model: application of a miniature extracorporeal circuit composed of asanguinous prime. *J Extra Corpor Technol*. 2005; 37 (1): 60–65.
13. *Ogura M, Yamamoto Y, Ogura Y.* A new simple temperaturecontrolled membrane oxygenator for the perfusion of isolated rat livers. *Experientia*. 1985; 41 (1): 139–141.
14. *Gourlay T, Ballaux PK, Draper ER, Taylor KM.* Early experience with a new technique and technology designed for the study of pulsatile cardiopulmonary bypass in the rat. *Perfusion*. 2002; 17: 191–198.
15. *Fabre O, Zegdi R, Vincentelli A et al.* A recovery model of partial cardiopulmonary bypass in the rat. *Perfusion*. 2001; 16 (3): 215–220.
16. *Houston RJ, de Lange F, Kalkman CJ.* A new miniature fiber oxygenator for small animal cardiopulmonary bypass. *Adv Exp Med Biol*. 2003; 540: 313–316.
17. *Bell RM, Mocanu MM, Yellon DM.* Retrograde heart perfusion: The Langendorff technique of isolated heart perfusion. *J Mol Cell Cardiol*. 2011; 50 (6): 940–950.
18. *DeCampos KN, Keshavjee S, Liu M, Slutsky AS.* Optimal inflation volume for hypothermic preservation of rat lungs. *J Heart Lung Transplant*. 1998; 17: 599–607.
19. *Noda K, Shigemura N, Tanaka Y, Bhama JK, D'Cunha J, Luketich JD, Bermudez CA.* Successful prolonged *ex vivo* lung perfusion for graft preservation in rats. *Eur J Cardiothorac Surg*. 2014; 45: 54–60.
20. *Cypel M, Yeung JC, Liu M, Anraku M, Chen F, Karolak W et al.* Normothermic *ex vivo* lung perfusion in clinical lung transplantation. *N Engl J Med*. 2011; 364: 1431–1440.

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COMPLEX USE OF PERFUSION TECHNIQUES IN KIDNEY TRANSPLANTATION FROM A DONOR WITH OUT-OF-HOSPITAL CARDIAC ARREST (CLINICAL CASE)

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Objective: to present the successful experience with a donor with out-of-hospital cardiac arrest (OHCA) in whom a set of modern perfusion techniques was used to obtain kidneys suitable for transplantation. **Materials and methods.** Automatic chest compression was resumed in an OHCA donor (after biological death has been confirmed in the hospital) to maintain minimal perfusion under mechanical ventilation with 100% FiO_2. With femoral vein cannulation, an extracorporeal circuit with a centrifuge pump and oxygenator was connected and abdominal normothermic regional perfusion was initiated. After 215 minutes, kidney was explanted under normothermic machine perfusion. Next, the left kidney was placed in the LifePort Kidney Transporter for hypothermic machine perfusion of donor kidneys. Perfusion time was 285 minutes. The right kidney was transplanted without additional ex-vivo perfusion. **Results.** Due to the complex use of perfusion techniques both in the donor body and ex-vivo, donor kidneys, after OHCA, with a total warm ischemia time of 110 minutes, were transplanted to recipients with good results. In the postoperative period, there was delayed function of the left and right renal grafts. The patients were discharged in a satisfactory condition under outpatient follow-up. **Conclusion.** The possibility and efficiency of organ donation after OHCA, facilitated by modern perfusion techniques and devices, open up a new perspective in addressing the organ shortage crisis.

Keywords: donors with out-of-hospital cardiac arrest, perfusion devices, kidney transplantation.

INTRODUCTION

Organ shortage crisis is a major public health problem. This has prompted the search for new solutions to increase the number of organ transplants. Patients with out-of-hospital cardiac arrest (OHCA) can constitute a very effective donor pool [1]. According to the modified Maastricht classification (Paris, 2013) [2], donors with OHCA are categorized as uncontrolled, IA (sudden OHCA without attempts at cardiopulmonary resuscitation), and IIA (sudden irreversible OHCA with ineffective cardiopulmonary resuscitation). The IIA donor category is most commonly used in clinical practice.

In 2013–2014, donors with OHCA class IIA accounted for about 64.2–54.1% of the total asystolic donation in Spain. With regulatory introduction of controlled organ donation, however, their proportion decreased to 15.6% in 2017 [2].

For Russia, this type of donation is undoubtedly relevant. In the first 10 years of the current century, there began to appear Russian publications concerning the beginning of the clinical use of automated chest compression (ACC) devices during cardiopulmonary resuscitation (CPR) [3, 4]. Among the advantages of these devices over manual CPR was the possibility of using them in OHCA conditions, primarily to achieve high-quality CPR when transporting a patient to the hospital. The current state of organ donation in Moscow, combined with the technical capabilities and experience of the Moscow Organ Donation Coordinating Center at Botkin Hospital, allow us to develop our own protocol for working with donors with OHCA and ensure its proper organization.

A clinical case study of OHCA donor management is presented in this publication.

CLINICAL CASE

From medical history. *A 33-year-old man was brought to the hospital in a state of clinical death with an incoming diagnosis of suspected pulmonary embolism (PE). Prehospital cardiac arrest. Acute heart failure. Acute respiratory failure. Artificial ventilation (AVL) at the prehospital stage.*

CPR was initiated by an emergency medical team using ACC devices. At the time of delivery to the hospi-

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tal, CPR had lasted for 53 minutes. In-hospital resuscitation lasted for 30 minutes, without effect. Biological death was confirmed. After death was confirmed, ACC devices together with AV (FiO_2 100%) were resumed to maintain perfusion of organs until the beginning of their preservation.

Preservation of abdominal organs under normothermic extracorporeal membrane oxygenation (nECMO). *A standard surgical access to the femoral vessels on the right side was performed. Using the Seldinger technique, 23 Fr (38 cm) and 19 Fr (23 cm) cannulas were inserted into the femoral vein and femoral artery, respectively, using an open method. On the left, a 16 Fr (90 cm) diameter double-balloon triple-lumen catheter (DBTL catheter) was placed into the femoral artery using an open method, and the thoracic balloon was inflated above the diaphragm level. The cannulas were retrogradely filled with donor blood and connected to an ECMO circuit. Abdominal normothermic perfusion was performed using Ex Stream (TransBioTech, Russia), an ECMO perfusion device. See Fig. 1.*

The perfusion temperature was maintained at 35 °C by means of a thermostat (Heater Unit HU 35, Maquet, Germany). Homeostasis was monitored by analyzing the acid-base state of arterial blood from the circuit at 1-hour intervals. The flow rate was maintained at ≥2.4 L/min. See Table 1.

During perfusion, solutions of alprostadil, furosemide, methylprednisolone, insulin, vancomycin hydrochloride or meropenem trihydrate were injected into the circuit. The required perfusion rate was achieved by infusing balanced crystalloid solutions into the extracorporeal circuit. Perfusion lasted for 215 minutes. With nECMO ongoing, the donor was transported to the operating room. See Fig. 2.

Kidney explantation for transplantation. *Median laparotomy was performed under nECMO. Warm scarlet blood was actively flowing from the edges of the surgical wound. Abdominal revision revealed no pathological effusion. The appearance of the abdominal organs (color, blood filling) corresponds to that in the case of organ*

Table 1

Acid-base status of donor arterial blood at the time of death and during nECMO

Acid-base status parameters	Death pronouncement	Hour 1 of perfusion	Hour 2 of perfusion
pH	6.61	7.031	7.772
pO_2 (mmHg)	63.1	595.6	666.2
pCO_2 (mmHg)	93.2	10.0	12.2
K^+ (mmol/L)	5.4	6.56	6.51
Na^+ (mmol/L)	148.0	133.3	142.6
BE (mmol/L)	–30		–1.34
Hemoglobin (g/L)	136	98.3	58.9
Glucose (mmol/L)	26.3	19.4	17.9
Lactate (mmol/L)	20.0	20.0	17.9
Urea (mmol/L),	6.3	–	–
Creatinine (μmol/L)	139	–	–

Fig. 1. Ex Stream, a perfusion device for extracorporeal membrane oxygenation

Fig. 2. Donor transportation (maintained by nECMO) to the operating room

explantation from a brain-dead donor. The organs were warm on palpation. Small bowel peristalsis was noted. A container with organ preservation solution "Custodiol" cooled to +4 °C was connected to the port in the venous part of the circuit, the preserving solution started to flow into the circuit and further through the oxygenator to abdominal organs. To drain the preserving solution after passage through the abdominal organs, a part of the venous circuit up to the port where Custodiol enters was isolated with clamps, the line was crossed, the free end was placed in a container to collect the drain. The rate of Custodiol inflow was 500.0 mL/min, washing to "clean water". In parallel with washing the organs with a preservative solution, sterile ice chips were placed in the abdominal cavity for local cooling. According to the standard technique, the right and left kidneys with vascular elements – a fragment of the aorta and inferior vena cava – were isolated and removed as a single block. The kidneys were separated on the table and examined in detail. On examination, the left renal graft was found to be of medium size, homogeneously colored, without tumor-like formations, with a single renal artery extending from the aorta and with a single renal vein. The right kidney graft was medium-sized, homogeneously colored, with a small cystic mass, there were two renal arteries branching from the aorta and a single renal vein. See Fig. 3.

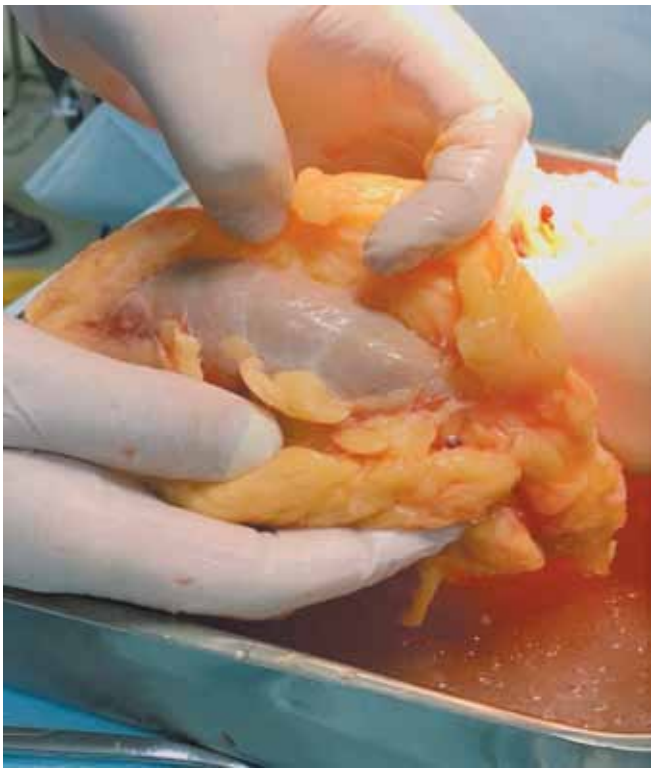

Fig. 3. Left donor kidney after explantation

Fig. 4. External view and baseline kidney perfusion parameters in the LifePort Kidney Transporter

Kidneys obtained from donors after OHCA have an increased risk of primary dysfunction or delayed function after transplantation because total warm ischemia time in this type of donation reaches critical values, up to 150 minutes. To reduce additional ischemic injury in kidneys recruited from expanded criteria donors and donors with irreversible circulatory arrest during static cold preservation, it is suggested to replace the latter partially/completely by perfusion preservation of donor kidneys provided by mechanical circulation of the perfusion solution through the donor kidney at different temperature regimes (hypothermic, normothermic) and possible oxygenation of the perfusion solution. Machine perfusion techniques have become an important tool in solving critical problems of organ transplantation, such as ischemia-reperfusion injury [5–7], unsatisfactory post-transplant graft function and lower graft survival [8, 9].

The experience of using commercially available machines for donor kidney perfusion in Russia is extremely limited, and there is no experience at all for kidney perfusion from a donor with OHCA [10]. At Botkin Hospital, it is possible to perform hypothermic machine perfusion of donor kidneys on the LifePort Kidney Transporter machine (Organ Recovery Systems, USA); accordingly, the left kidney graft sent to Botkin Hospital was placed in the mentioned machine for kidney perfusion. See Fig. 4.

Perfusion temperature did not exceed 8 °C. Perfusion pressure at the start of perfusion was 20/17 mmHg, taking into account pulsatile perfusion mode, with a flow rate of 94 ml/min and a resistivity index (RI) of 0.19 mmHg/mL/min. Machine perfusion lasted for 285 minutes. At the time of completion of donor kidney perfusion, perfusion pressure decreased to 10/3 mmHg, while the flow rate remained quite high, 79 ml/min, and the RI, which is calculated taking into account the above two parameters, decreased to 0.07 mmHg/mL/min. See Fig. 5.

The right kidney graft was sent to a transplant center where ex-vivo machine perfusion was not performed.

Recipients. *The donor-recipient pair was selected taking into account cross-match test and HLA compatibility results.*

Left kidney transplant recipient. *A 39-year-old woman, end-stage chronic kidney disease (CKD), diabetic nephropathy. Renal replacement therapy – hemodialysis, since February 11, 2017. Five HLA mismatches with the donor. On the waiting list since May 10, 2018. Kidney transplantation was performed on March 21, 2023. Surgery lasted from 05:50 to 09:25. After initiating blood flow at 07:47, the graft acquired physiological turgor, uniformly turned pink, and urine flow was noted. As the graft warmed up, pulsation of renal arteries was satisfactory, patency of vascular anastomoses was not impaired. After suturing the muscles of the anterior abdominal wall, an ultrasound examination was performed in the operating room, which found blood flow in the transplanted kidney to be satisfactory and resistivity index to be 0.50. See Fig. 6.*

Fig. 5. Final perfusion parameters

There was delayed graft function in the postoperative period. Therefore, five hemodialysis sessions were performed. At the moment of discharge from the clinic on day 36, urea and creatinine levels were 19 mmol/L and 117 μmol/L, respectively, diuresis was 1300 mL per day. See Table 2.

Right kidney transplant recipient. *A 51-year-old woman, end-stage CKD. Four HLA mismatches with the donor. Renal replacement therapy in the form of hemodialysis since September 22, 2008. On the waiting list since January 9, 2020. Kidney transplantation performed on March 21, 2023. After blood flow started, the graft acquired physiological turgor and color, urine flow was visualized along the ureter. On day 1 after the operation, 1700 mL diuresis was noted, which decreased on day 2. In the postoperative period, 3 hemodialysis sessions were performed. At the time of discharge on day 16, blood urea was 21 mmol/L, creatinine was 250 μmol/L, and diuresis was 3200 mL per day. See Table 2.*

DISCUSSION

OHCA is a major public health problem in both Europe and the United States. There are approximately 275,000 cardiac arrests annually and approximately 420,000 in the United States. Detailed epidemiologic information on this problem was, for the first time, presented in the international prospective multicenter study EuReCa ONE, which pooled data from 27 countries. The population of this study is represented by 10,682 cases of confirmed OHCA, of which 7,146 involved CPR. Of all patients hospitalized with OHCA, 25.2% had sponta-

Fig. 6. Doppler ultrasound of the left renal graft at the completion of surgery

neous circulatory recovery, 10.7% had continued CPR in the hospital, and 64.0% of patients were confirmed dead at the time of admission [11]. The high mortality rate of OHCA patients is noteworthy – according to EuReCa data, the survival rate among all patients who had CPR does not exceed 10.3%.

There is little Russian data on OHCA. Birkun A.A. (2017) points to the high prevalence of OHCA in a single administrative center of the Russian Federation, an order of magnitude higher than in many foreign countries [12]. The Center for the treatment of sudden cardiac death at Pavlov University provides data showing that there is extremely high mortality of patients with OHCA, which reached 92.6% [13].

The above foreign and Russian data suggest that there is a high proportion of potential donors among OHCA patients.

From the logistical and technological points of view, organ donation after OHCA is among the most challenging. Organizing this type of donation in a similar manner to leading foreign protocols, mainly from Spain and France, requires significant human and technical resources, which in turn raises the question of expediency and efficiency of the technologies under consideration. The most reliable data on outcomes of transplantations from such donors are presented by the corresponding programs in Spain, France, and Italy. The proportion of donors with OHCA from whom at least one organ was transplanted does not exceed 80.0% [14]. Poor preservation, associated with critical warm ischemia time, is the most common reason for not utilizing organs for transplantation [15]. However, outcomes of organ transplantation from donors with OHCA are considered acceptable, although with room for improvement. Kidney transplants have comparable short-term and long-term outcomes despite a higher incidence of primary dysfunction and delayed function compared with organs from brain-dead donors and controlled cardiac arrest donors [16–20].

The leading risk factor in organ donation with OHCA is the critical value of warm ischemia time. Normothermic regional perfusion significantly reduces the risk of graft dysfunction and is crucial for achieving optimal outcomes in kidney transplantation from such donors [19, 21]. However, even with careful donor selection and the use of normothermic regional perfusion, incidence of primary kidney graft dysfunction is 7–8% [22].

Because renal grafts from donors with OHCA are subject to prolonged and repeated ischemic injury, it is important to assess their viability before transplantation based on functional, anatomical and histological data, including *ex-vivo* machine perfusion [23].

Significant factors in evaluating a potential donor with OHCA are the time from the moment of cardiac arrest (the exact time can be known only if there are witnesses to this event) to the start of CPR (this time should not exceed 30 minutes for possible kidney donation and 15 minutes for possible liver donation) and the total warm ischemia time determined from cardiac arrest to initiation of organ preservation (this time should not exceed 150 minutes). As these time intervals increase, the risk of getting a non-functioning graft is significantly higher.

In the presented clinical case, the total warm ischemia time amounted to 110 minutes, which falls within the

Table 2

Characteristics of left and right kidney recipients

Characteristics	Left kidney recipient	Right kidney recipient
Gender (male/female)	Female	Female
Age (years)	39	51
Diagnosis	Stage 5 CKD, diabetic nephropathy	Chronic glomerulonephritis, stage 5 CKD
Date of hemodialysis initiation	February 11, 2017	September 22, 2008
Number of HLA-A, B, Dr mismatches	5	4
Length of stay on the waiting list (months)	57	38
Ex-vivo machine perfusion	Yes	No
Total cold ischemia time (hour)	17.7	21.3
Resistance index (RI) at end of surgery	0.50	–
RI on day 1	0.74	0.86
RI on day 7	0.7	0.80
RI at the time of discharge	0.80	1.0
Graft function	Delayed	Delayed
Number of post-transplant hemodialysis sessions	5	3
Urea/creatinine levels on day 1 (mmol/L) (μmol/L)	36/705	43/800
Urea/creatinine levels on day 7	37/381	24/360
Urea/creatinine levels at the time of discharge	19/117	21/250
Inpatient stay (bed days)	36	16

above-mentioned time limits. We consider the obtained outcomes, despite development of delayed renal graft function in recipients, to be satisfactory and comparable with foreign experience. Thus, incidence of delayed graft function for kidneys obtained from donors with OHCA is about 50–70%. Nevertheless, the authors note that such grafts have satisfactory 1-year, 5-year, and even 10-year survival rates [19, 24–27].

It should be noted that the paper presented is the first Russian experience of using *ex-vivo* machine perfusion of a kidney obtained from a donor with OHCA, in whom extracorporeal normothermic regional perfusion was used. *Ex-vivo* hypothermic machine perfusion of renal grafts allows us to obtain an objective assessment of organ transplantability through the renal resistive index (RRI).

Our experience of *ex-vivo* kidney perfusion is at the initial stage, and it is important to take into account many years of similar experience from leading foreign donor programs. Analysis of 302 *ex-vivo* hypothermic perfused donor kidney transplants, including kidneys from donors after circulatory death, showed that RRI is an independent risk factor for delayed graft function and graft dysfunction in the first year after transplantation. Hence, RRI can be considered as an additional tool for evaluation of kidney grafts, especially high-risk ones. However, the low prognostic value of RRI limits its isolated use in deciding whether to use or dispose of high-risk donor kidneys for transplantation [28]. In an analysis of 336 consecutive machine perfusion procedures of donor kidneys from expanded criteria donors, Mozes et al. showed that the transplant outcomes of kidneys with an unfavorable range of 0.40 mmHg/mL/min < RRI < 0.60 mmHg/mL/min were similar to those of kidneys with more acceptable perfusion rates [29]. I. Jochmans et al. also point out the need for cautious interpretation of RRI. In a cohort of 302 kidney transplants that received hypothermic machine perfusion, the RRI of donor kidneys with primary dysfunction was comparable to the RRI of kidneys with immediate and delayed function after transplantation. In a retrospective analysis of the above-mentioned cohort of transplanted kidneys, none of the kidney transplant cases with an RRI >0.40 mmHg/mL/min reported primary nonfunction [30].

In the clinical observation under consideration, *ex-vivo* hypothermic perfusion of one of the kidneys lasted 4.75 hours. Here, it is important to note that the RRI index, which was 0.19 at the beginning of perfusion, indicating that the donor kidney was in good condition and that it was suitable for transplantation, decreased to 0.07 by the end of perfusion. This allowed us to confidently recommend this kidney for transplantation. At the same time, similar to data from foreign publications given above, the RRI does not have a high prognostic ability, since there was delayed graft function in the postoperative period, when at such low RRI, we could expect immediate function. However, it is impossible not to note the practically reference blood urea and creatinine levels at the time of discharge of the patient who received the graft after *ex-vivo* hypothermic perfusion in the LifePort device.

It seems extremely important to further accumulate data on *ex-vivo* kidney perfusion parameters to form our own idea of the relationship between machine perfusion parameters and immediate and long-term kidney transplant outcomes.

CONCLUSION

Moscow city is developing its own practice of working with OHCA donors. Effective combination of the established organizational model of organ donation for transplantation in Moscow and modern perfusion technologies provided the very possibility of working with such a complex category of donors and laid serious prerequisites for further development in this direction. This, in turn, will significantly increase the number of transplants in Moscow and provide valuable scientific knowledge about organ donation where warm ischemia time reaches critical levels.

The authors declare no conflict of interest.

REFERENCES

1. *Renaudier M, Binois Y, Dumas F, Lamhaut L, Beganton F, Jost D et al*. Paris Sudden Death Expertise Center group. Organ donation after out-of-hospital cardiac arrest: a population-based study of data from the Paris Sudden Death Expertise Center. *Ann Intensive Care.* 2022; 6; 12 (1): 48.
2. *Thuong M, Ruiz A, Evrard P, Kuiper M, Boffa C, Akhtar MZ*. New classification of donation after circulatory death donors' definitions and terminology. *Transpl Int.* 2016; 29 (7): 749–759.
3. *Shabunin AV, Minina MG, Drozdov PA, Sevostianov VM, Nesterenko IV, Makeev DA, Zhuravel OS.* Asystole kidney donation using automated chest compression system and hypothermic oxygenated machine perfusion (first experience in the Russian Federation). *Russian Journal of Transplantology and Artificial Organs*. 2022; 24 (2): 102–107.
4. *Reznik ON, Skvortsov AE, Reznik AO, Ananyev AN, Tutin AP, Kuzmin DO, Bagnenko SF.* Uncontrolled Donors with Controlled Reperfusion after Sixty Minutes of Asystole: *A Novel Reliable Resource for Kidney Transplantation*, Published: 2013; 30 (5).
5. *Zhong Z, Hu Q, Fu Z.* Increased Expression of Aldehyde Dehydrogenase 2 Reduces Renal Cell Apoptosis During Ischaemia/Reperfusion Injury After Hypothermic Machine Perfusion. *Artif Organs.* 2016; 40: 596–603.
6. *Yang Z, Zhong Z, Li M.* Hypothermic machine perfusion increases A20 expression which protects renal cells against Ischaemia/reperfusion injury by suppressing inflammation, apoptosis and necroptosis. *Int J Mol Med.* 2016; 38: 161–171.

7. *Chatauret N, Coudroy R, Delpech PO.* Mechanistic analysis of nonoxygenated hypothermic machine perfusion's protection on warm ischemic kidney uncovers greater enos phosphorylation and vasodilation. *Am J Transplant*. 2014; 14: 2500–2514.
8. *Moers C, Pirenne J, Paul A, Ploeg RJ.* Machine Preservation Trial Study Group. Machine perfusion or cold storage in deceased-donor kidney transplantation. *N Engl J Med.* 2012; 366: 770–771.
9. *Jochmans I, Moers C, Smits JM.* Machine Perfusion Versus Cold Storage for the Preservation of Kidneys Donated After Cardiac Death. *Ann Surg*. 2010; 252: 756–764.
10. *Reznik ON.* Organizatsionnye i tekhnologicheskie osnovy polucheniya i selektsii pochek dlya transplantatsii: avtoref. dis. … d-ra med. nauk. M., 2008; 47.
11. *Gräsner JT, Lefering R, Koster RW, Masterson S, Böttiger BW, Herlitz J et al.* EuReCa ONE Collaborators. EuReCa ONE-27 Nations, ONE Europe, ONE Registry: A prospective one month analysis of out-of-hospital cardiac arrest outcomes in 27 countries in Europe. *Resuscitation.* 2016 (12) 109: 145–146.
12. *Birkun AA, Glotov MA.* Epidemiologicheskie pokazateli vnegospital'noy ostanovki krovoobrashcheniya na primere otdel'no vzyatogo administrativnogo tsentra Rossiyskoy Federatsii. *Anesteziologiya i reanimatologiya*. 2017; 62 (2): 113–117.
13. *Teplov VM, Prasol DM, Kolomoytsev VV, Arkhangelsky ND, Bagnenko SF.* The center for the treatment of sudden cardiac death: the results of five years of work. *Messenger of Anesthesiology and Resuscitation*. 2023; 20 (1): 17–23.
14. *Dominguez-Gil B, Haase-Kromwijk B, Van LH, Neuberger J, Coene L, Morel P et al*. Current situation of donation after circulatory death in European countries. *Transpl Int*. 2011; (24): 676–686.
15. *Del Río F, Andrés A, Padilla M et al*. Kidney transplantation from uncontrolled donors after circulatory death: the Spanish experience. *Kidney Int*. 2019; 95: 420–428.
16. *Dominguez-Gil B, Duranteau J, Mateos A, Nunez JR, Cheisson G, Corral E et al.* Uncontrolled donation after circulatory death: European practices and recommendations for the development and optimization of an effective programme. *Transpl Int.* 2016; 29: 842–859.
17. *Minambres E, Rubio JJ, Coll E, Dominguez-Gil B.* Donation after circulatory death and its expansion in Spain. *Curr Opin Organ Transplant*. 2018; 23: 120–129.
18. *Delsuc C, Faure A, Berthiller J, Dorez D, Matillon X, Meas-Yedid V et al.* Uncontrolled donation after circulatory death: comparison of two kidney preservation protocols on graft outcomes. *BMC Nephrol.* 2018; 19 (1): 3.
19. *Demiselle J, Augusto JF, Videcoq M, Legeard E, Dube L, Templier F et al.* Transplantation of kidneys from uncontrolled donation after circulatory determination of death: Comparison with brain death donors with or without extended criteria and impact of normothermic regional perfusion. *Transpl Int.* 2016; 29 (4): 432–442.
20. *Molina M, Guerrero-Ramos F, Fernandez-Ruiz M, Gonzalez E, Cabrera J, Morales E et al.* Kidney transplant from uncontrolled donation after circulatory death donors maintained by nECMO has long-term outcomes comparable to standard criteria donation after brain death. *Am J Transplant*. 2018; 27: 5.
21. *Antoine C, Savoye E, Gaudez F, Cheisson G, Badet L, Videcoq M et al.* Kidney Transplant From Uncontrolled Donation After Circulatory Death: Contribution of Normothermic Regional Perfusion, National Steering Committee of Donation After Circulatory Death. *Transplantation.* 2020; 104 (1): 130–136.
22. *Summers DM, Pettigrew JG.* Kidney transplantation following uncontrolled donation after circulatory death. *Curr Opin Organ Transplant*. 2020; 25 (2): 144–150.
23. Organizacion Nacional de Trasplantes Donación en asistolia en España: situación actual y recomendaciones. Documento de Consenso 2012. http://www.ont.es/infesp/pdf Accessed October 16, 2018.
24. *Barrou B, Billault C, Nicolas-Robin A*. The use of extracorporeal membranous oxygenation in donors after cardiac death. *Curr Opin Organ Transplant*. 2013; 18 (2): 148–153.
25. *Abboud I, Viglietti D, Antoine C, Gaudez F, Meria P, Tariel E et al.* Preliminary results of transplantation with kidneys donated after cardiocirculatory determination of death: A French single-centre experience. *Nephrology, Dialysis, Transplantation*. 2011; 27 (6): 2583–2587.
26. *Lee JH, Hong SY, Oh CK, Hong YS, Yim H.* Kidney transplantation from a donor following cardiac death supported with extracorporeal membrane oxygenation. *Journal of Korean Medical Science.* 2012; 27 (2): 115–119.
27. *Molina M, Guerrero-Ramos F, Fernandez-Ruiz M, Gonzalez E, Cabrera J, Morales E et al.* Kidney transplant from uncontrolled donation after circulatory death donors maintained by nECMO has long-term outcomes comparable to standard criteria donation after brain death. *Am J Transplant.* 2019; 19 (2): 434–447.
28. *Moers JC, Smits JM.* The Prognostic Value of Renal Resistance During Hypothermic Machine Perfusion of Deceased Donor Kidneys. *Am J Transplant.* 2011; 11: 2214–2220.
29. *Mozes MF, Skolek RB, Korf BC.* Use of perfusion parameters in predicting outcomes of machine-preserved kidneys. *Transplant Proc.* 2005; 37: 350–351.
30. *Jochmans I, Moers C, Smits JM, Leuvenink HGD, Treckmann J, Paul A et al*. The Prognostic Value of Renal Resistance During Hypothermic Machine Perfusion of Deceased Donor Kidneys. *Am J Transplant.* 2011; 11 (10): 2214–2220.

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SURGICAL TECHNIQUE FOR EXPLANTATION OF A FUNCTIONING CARDIOPULMONARY COMPLEX IN AN EXPERIMENT

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Objective: to develop and approve the surgical technique for explantation of a functioning cardiopulmonary complex under normothermic autoperfusion. **Materials and methods.** Landrace pigs were used as the experimental model for a series of acute experiments (n = 10). During the experiment, invasive pressure in the cavities of the heart and main arteries, blood gas composition, and myocardial contractility were monitored. The functioning cardiopulmonary complex was explanted through a median sternotomy. The explanted complex was conditioned at 37–38 °C for 6 hours. **Results.** In the course of a series of experiments, it was shown that stable operation of the isolated heart-lung complex *ex vivo* for 6 hours was fundamentally possible provided that the parameters of the basic homeostasis constants are maintained. The technological solutions used made it possible to ensure safe hemodynamic and anatomical isolation of the working cardiopulmonary complex. **Conclusion.** The developed protocol for isolating a functioning cardiopulmonary complex allows to provide stable graft function for 6 hours under normothermic autoperfusion. Implementation of this concept in the development of transport systems would significantly facilitate their design and eliminate the use of expensive components. This would contribute to widespread introduction into clinical practice.

Keywords: chronic heart failure, heart transplantation, heart preservation, autoperfusion, donor organ preservation, ex vivo organ perfusion.

INTRODUCTION

Solid organ transplantation is undoubtedly one of the most significant achievements in medicine in the 20th century. However, many problems in this field still remain unresolved [1]. One of such problems is the development of technology for long-term conditioning of donor organs. The safe time limit of cold preservation of the heart remains the main constraint that does not allow expanding the geography of donor bases [2, 3]. Given the obvious advantages of normothermic conditioning of donor organs over static cold preservation, most current graft preservation strategies focus on maintaining blood flow and temperature control [4, 5]. Instead of cooling the organ to slow down metabolic processes, machine perfusion maintains normal metabolic activity under conditions close to the physiologic environment. This allows to significantly reduce cold ischemia time or abandon it altogether when implanting an organ into the recipient's body, as well as to perform extended screening of the morphofunctional status of the graft [4, 6–8]. However, widespread use of such perfusion platforms in many countries is limited by high cost [9–13]. In this regard, the development of an effective and cost-effective method of long-term conditioning of donor hearts is an urgent problem for modern transplantology.

Long-term studies of the physiological laws of cardiac autoregulation have traditionally been associated with the development of methods for long-term maintenance of effective cardiac activity *ex vivo* [14–16]. And although at that time, the ideas of experimental physiologists about transplantology were very far from the modern ones, today the realization of the concept of autonomous survival of donor organs under conditions of normothermic autoperfusion can become a solution to the problem of long-term conditioning of graft, significantly simplify the development of machine perfusion platforms and contribute to widespread introduction of these technologies into clinical practice.

MATERIALS AND METHODS

Female Landrace pigs, weighing 50 ± 5 kg, aged 4–5 months, were used as an experimental model for a series of acute experiments (n = 10). Care, mainte-

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nance of the experiment, observation and withdrawal of animals from it were performed in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasbourg, March 18, 1986) and were approved by the Bioethics Committee at Meshalkin National Medical Research Center (Protocol No. 2 of September 1, 2022).

On the day of the experiment, all animals were premedicated (Zoletil 100) on an empty stomach. The dose was selected individually, based on weight and height parameters. After the onset of sleep, the surgical field and the area of catheterization of neck vessels were prepared. Then the animal was transported to the operating table and fixed in a supine position for subsequent tracheal intubation, installation of central arterial and venous catheters into the external jugular and common femoral vein. The experiment was performed under endotracheal anesthesia with sevoflurane and myorelaxation (rocuronium bromide). Artificial ventilation (AV) was performed using an anesthetic breathing machine Fabius Plus (Draeger, FRG) with positive pressure on inhalation (20–30 cm of water column) and on exhalation (5–8 cm of water column), with a respiratory volume of 8 ml/kg, with a frequency of 12–14 breaths per minute. Vital parameters were recorded using an IntelliVue MP70 monitor (Philips, Netherlands).

During the experiments, we monitored invasive blood pressure (IBP) by catheterization of the right common carotid artery, central venous pressure (CVP) by catheterization of the right external jugular vein, and blood gasses. Blood analysis was performed using an automated hematology analyzer XT-4000i (Sysmex, Germany) according to the manufacturer's guidelines. Central hemodynamics were investigated by catheterization of the right heart with a Swan–Ganz catheter, as well as using a portable multifunctional ultrasound system Philips CX50 (Philips Ultrasound, USA) with ECG synchronization.

Explantation of the functioning cardiopulmonary complex (fCPC) was performed through median sternotomy. Isolation of fCPC was initiated with mobilization of the superior vena cava (SVC) and ligation of the unpaired vein. Then the brachiocephalic trunk (BCT), both carotid arteries, and the left subclavian artery (LSCA) were isolated. The trachea was carefully separated from the esophagus using an electrocoagulator, achieving thorough hemostasis. Particular attention was paid to the release of the lower lung lobes, since the basal lung sections are extremely deep and mostly covered by the diaphragm dome, which makes visualization difficult and carries a high risk of surgical trauma to the parenchyma.

After administration of heparin (3 mg/kg body weight), LSCA was ligated and transected, avoiding rough traction. A 16–18 Fr arterial cannula was inserted through the right subclavian artery toward the heart and connected to a reservoir suspended 70 cm above the heart. The semi-unpaired vein draining blood in the animals directly into the coronary sinus was ligated and transected. Under IBP control, all brachiocephalic arteries were ligated, avoiding pressure increase of more than 130–140 mmHg in the aortic root due to dosed exfusion of blood into the reservoir. After clamping the descending thoracic aorta at the level of the isthmus, arterial blood was withdrawn into the reservoir until the blood level stabilized. After stabilization of blood level and arterial pressure, 1–1.5 liters of Ringer's solution was injected into the femoral vein. Then the vena cava was ligated and transected, the trachea was transected and reintubated with a cuffed tube, the fCPC was finally separated from the surrounding tissues, transferred to a container with warm saline (38°C) and observation continued.

Statistical data processing was performed using Statistica 10.0 software (StatSoft Inc., USA). Normality of distribution was checked using the Shapiro–Wilk test with subsequent assessment of equality of variances by Levene's test. In the case when distribution in the experimental groups was normal and there was intergroup equality of variance, further processing was carried out using parametric statistics, the Student's t test. When the distribution is different from the normal, nonparametric statistics were used. Differences between the parameters were considered reliable at $p < 0.05$.

RESULTS

In a series of acute experiments, 10 fCPC explantations were performed with 6-hour follow-up (Fig. 1).

Active reservation of the animals' own blood through an arterial cannula placed in the brachiocephalic trunk, and displacement of the maximum volume of blood from the lower half of the body by infusion of crystalloid solution into the femoral vein allowed to create the necessary blood supply (1500–2000 mL) to maintain normovolemia in fCPC during 6 hours of follow-up. An arterial blood reservoir, suspended at a height of 70 cm above the heart level, provided stable conditions for transfer of isolated fCPC into the transport container, acting either as a receiver for its own cardiac output or providing antegrade coronary perfusion according to the Langendorff technique (Fig. 2). After placement of fCPC in a container and installing measuring sensors, the arterial trunk was clamped (Fig. 2, b), then the blood volume in the fCPC was adjusted under pressure control in the heart cavities.

The main hemodynamic parameters were measured using a Philips CX50 ultrasound system (Philips Ultrasound, USA) with ECG synchronization, as well as direct tonometry in the heart cavities and main arteries (Table 1).

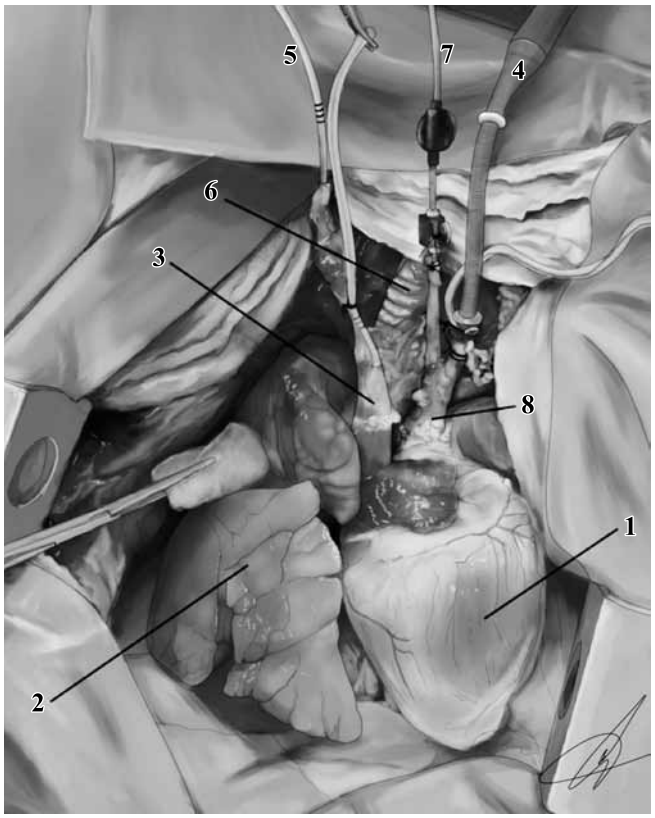

Fig. 1. General view of surgical wound; 1, heart; 2, right lung; 3, superior vena cava; 4, arterial cannula; 5, Swan–Ganz catheter; 6, trachea; 7, arterial catheter; 8, ascending aorta

To maintain the main homeostasiologic constants for 6 hours of normothermic autoperfusion, calcium chloride and glucose were infused into the right heart sections. fCPC ventilation was performed using anesthesia-breathing apparatus FabiusPlus (Draeger, FRG) with positive inspiratory pressure (20–30 cm of water column) and expiratory pressure (5–8 cm of water column) with respiratory volume of 8 mL/kg of body weight, frequency of 12–14 breaths per minute, FiO_2 – 70%. The main parameters of blood gas composition are shown in Table 2.

DISCUSSION

The need for research into the functioning of an isolated heart and the cardiopulmonary complex was fully recognized more than a century ago. In 1866, at the Carl Ludwig Physiological Institute in Leipzig, C. Elias described the effect of diastolic filling in an isolated perfused frog heart on cardiac output [15]. Later, a study describing the effect of filling pressure on contraction amplitude was published by C. Joseph in 1869 [16]. In 1881, H.N. Martin described the technique of preparing a hemodynamically isolated cardiopulmonary complex of a dog with an open chest using a resistor and a reservoir between the aorta and vena cava [17]. Thin-walled tubes, surrounded by a sealed cylinder (similar to modern hemodialysis columns), were used as a resistor. This

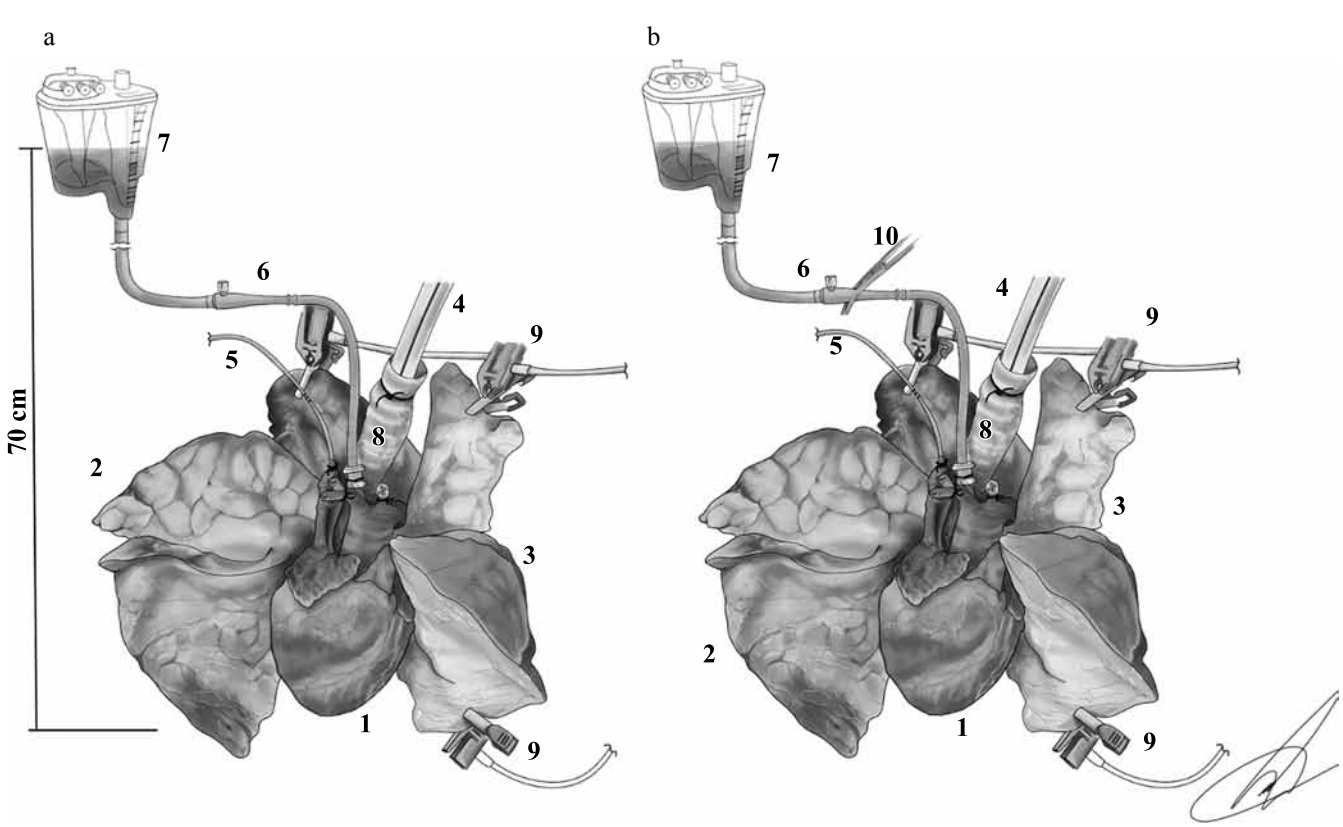

Fig. 2. Diagram of the isolated cardiopulmonary complex: a, stage of blood exfusion into the reservoir and preparation for transfer of the complex into the container; b, stage of final hemodynamic isolation of a working cardiopulmonary complex; 1, heart; 2, right lung; 3, left lung; 4, intubation tube; 5, Swan–Ganz catheter; 6, arterial cannula; 7, blood reservoir; 8, trachea; 9, electrocardiograph electrodes; 10, clamp

drug was used to study the contractile properties of the heart, cardiac metabolism, regulation of coronary blood flow and the effect of various pharmacological drugs [18–20]. Another widely known method of maintaining heart function after anatomical isolation was proposed in 1895 by O. Langendorff [21]. The developed method involved retrograde injection of saline into the aortic root. At the same time, it was proved that the heart could function for a long time thanks to the consumption of oxygen dissolved in saline solution. However, despite the absence of external work of the emptied left ventricle of the heart under retrograde Langendorff perfusion conditions, the use of saline significantly limited the survival time of the heart. Since meeting the myocardial oxygen demand required increased coronary flow of crystalloid solution, this inevitably led to myocardial edema and deterioration of its contractility. Enrichment of the solution by addition of washed red blood cells restored the oxygen content and viscosity of the perfusate, which helped to reduce the resistance of the capillary coronary bed.

Another modification that made the Langendorff preparation more practical was the scheme that included a reservoir filled with perfusate under constant pressure and connected both to the left atrium and aorta through a system of valves and artificial vascular resistance [22]. At the same time, blood flowing from the coronary sinus was reserved and excluded from recirculation. Thanks to this scheme, the left ventricle generated cardiac output by doing external work under controlled filling pressure conditions. A similar type of cardiac isolation was proposed by G. Elzinga [18].

In 1926, E.H. Starling and M.B. Visscher published the results of studies of an isolated heart, formulating the well-known law describing the relationship between diastolic heart volume and the force of heart contractions [14, 23]. At the same time, historically, Starling's cardiac preparation was not subjected to as many modifications as Langendorff's scheme.

A work by P.H. Huisman et al. presents a description of a modified Starling preparation, which was developed to study ventricular electrical activation and then adapted to study the valve apparatus and ventricular function and mechanics [24]. The P.H. Huisman method allowed to ensure a long period of stable mechanical work of

Table 1

Main hemodynamic parameters

Parameter	Hour 1	Hour 2	Hour 3	Hour 4	Hour 5	Hour 6
HR (bpm)	66 [51; 95]	94 [90; 100]	97 [87; 105]	93 [86; 97]	89 [87; 92]	89 [89; 94]
RAP (mmHg)	0	–1	5	4	3	1
mRVP (mmHg)	7 [3; 12]	8.3 [6.5; 11]	10.6 [8.7; 12]	6 [5; 7]	4.3 [3; 5.5]	5.5 [3; 7.5]
mPAP (mmHg)	6.5 [3.5; 10]	5.2 [3.5; 8.5]	10.5 [10; 11]	6.8 [4; 9]	5.2 [4; 6]	7.5 [4.7; 9.7]
PWP (mmHg)	1	0	6	2	1	4
IBP in aorta (mmHg)	67 [54; 74]	75 [65; 85]	93 [89; 101]	85 [70; 100]	73.5 [64.5; 85]	70.8 [62.5; 78]
CO (L/min)	903.0	846.0	1015.0	1089.0	1414.0	899.0

Note: HR, heart rate; RAP, Right Atrial Pressure; mRVP, Mean Right Ventricular Pressure; MPAP, Mean Pulmonary Artery Pressure; PWP, pulmonary wedge pressure; IBP, invasive blood pressure; CO, cardiac output. Data is presented as Me [Q1; Q3].

Table 2

Main parameters of blood gas composition

Parameter	Hour 1	Hour 2	Hour 3	Hour 4	Hour 5	Hour 6
Hematocrit (%)	24.5	29.8	28.5	26.0	27.0	27.6
Hemoglobin (g/L)	79	79	78	69	87	90
pH	7.9	7.8	7.8	7.8	7.7	7.7
Lactate (mmol/L)	6.3	8.4	5.3	1.5	2.1	3.6
Glucose (mmol/L)	7.7	11.1	8.7	3.5	5.9	3.3
Aortic PaO_2 (mmHg)	248	170	190	197	238	175
Right atrial PaO_2 (mmHg)	39.6	40.3	42.8	31.4	34.7	31.2
Left atrial PCO_2 (mmHg)	5.7	5.6	6.7	6.7	8.4	6.9
PaO_2/FiO_2	2.8	2.6	2.71	2.8	3.4	2.5
K^+ (mmol/L)	3.8	3.3	2.2	2.8	3.1	3.0
Na^+ (mmol/L)	143	147	152	155	160	163
Ca^{++} (mmol/L)	0.72	0.77	1.18	1.34	0.87	1.39

Note: PaO_2, arterial partial pressure of oxygen; PCO_2, partial pressure of carbon dioxide; FiO_2, fraction of inspired oxygen; K^+, potassium; Na^+, sodium; Ca^{++}, calcium.

the heart largely due to the use of whole, practically undiluted fresh blood as perfusate. Another important technique was the preservation of the anatomic integrity of the anastomosis between the pulmonary veins and the left atrium, which ensured normal filling of the left ventricle. The authors also corrected the weaknesses of the original Starling technique by performing complete denervation of the cardiopulmonary complex.

Fundamental knowledge gained during these studies formed the basis of modern technologies for prolongation of cardiac graft survival *ex vivo*. However, the principle of autonomous survival of an isolated cardiopulmonary complex remains unrealized in any of the existing models of donor organ transport modules [14, 24–26]. Previous experiments made it possible to identify several critical requirements necessary for successful isolation and long-term functioning of fCPC, among which are compliance with the principles of saving the donor blood volume for subsequent correction of the level of volemia and the possibility to effectively maintain the normothermic graft conditioning mode [27]. During the study, the effectiveness of using active exfusion of donor blood at the expense of the donor's own cardiac output was shown. The combination of methods of functional isolation of fCPC and Langendorff perfusion elements made it possible to obtain the maximum possible blood volume, to ensure stable hemodynamic parameters at all stages of fCPC explantation and dosed volume loading of the complex. Stability of hemodynamic parameters and self-regulation of coronary blood flow due to the height of the blood reservoir location allow for the most careful fCPC dissection with meticulous hemostasis.

An important feature of isolated fCPC functioning is the ability to maintain sufficient coronary blood flow under absolute hypovolemia of pulmonary circulation. If in the case of anatomical integrity, the right ventricles actually determine the left ventricular flow rate, in the case of fCPC isolation, the left sections are in optimal conditions of pressure and volume load with minimal shock work of the right ventricle. So, despite maintenance of average level of arterial pressure in the aortic root at 65–75 mmHg, cardiac output ranged from 846.0 to 1414.0 mL per minute, with the presence of a pulse curve in the right ventricular cavity corresponding in characteristics to that before fCPC explantation at all stages of the experiment, there was complete absence of a pulse curve in the pulmonary artery trunk.

Such sparing conditions for autonomous functioning of fCPC provide "rest" to the right heart, allowing it to generate sufficient stroke volume with minimal afterload. It is important to note that these fCPC functioning conditions have much in common with those observed during active cardiac perfusion. However, the development of the transport system based on normothermic autoperfusion principles allows us to significantly reduce the economic costs of prosthetic pumping function of the heart and oxygenating function of the lungs. This will facilitate introduction of long-term conditioning of donor organs *ex vivo* into clinical practice.

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The authors declare no conflict of interest.

REFERENCES

1. *Fomichev AV, Poptsov VN, Sirota DA, Zhulkov MO, Edemskiy AG, Protopopov AV et al*. Mid-term and long-term outcomes following heart transplantation with prolonged cold ischemia. *Russian Journal of Transplantology and Artificial Organs*. 2023; 25 (1): 99–105. (In Russ.). https://doi.org/10.15825/1995-1191-2023-1-99-105.
2. *Pannekoek A, Ali U*. Does the solution used for cold static storage of hearts impact on heart transplant survival? *Interact Cardiovasc Thorac Surg*. 2021; 33 (5): 814–818. doi: 10.1093/icvts/ivab164.
3. *Fomichev AV, Khvan DS, Agaeva HA, Zhulkov MO, Doronin DV, Chernyavsky AM*. Experience of heart transplantation with an extended cold ischemic time of donor heart. *Russian Journal of Cardiology*. 2020; 25 (8): 4011. https://doi.org/10.15829/1560-4071-2020-4011.
4. *Qin G, Jernryd V, Sjöberg T, Steen S, Nilsson J*. Machine Perfusion for Human Heart Preservation: A Systematic Review. *Transpl Int*. 2022; 35: 10258. doi: 10.3389/ti.2022.10258.
5. *Peters-Sengers H, Houtzager JHE, Idu MM, Heemskerk MBA, van Heurn ELW, Homan van der Heide JJ et al*. Impact of Cold Ischemia Time on Outcomes of Deceased Donor Kidney Transplantation: An Analysis of a National Registry. *Transplant Direct*. 2019; 5 (5): e448. doi: 10.1097/TXD.0000000000000888.
6. Ontario Health (Quality). Portable Normothermic Cardiac Perfusion System in Donation After Cardiocirculatory Death: A Health Technology Assessment. *Ont Health Technol Assess Ser*. 2020; 20 (3): 1–90.
7. *Van Raemdonck D, Rega F, Rex S, Neyrinck A*. Machine perfusion of thoracic organs. *J Thorac Dis*. 2018; 10 (Suppl 8): S910–S923. doi: 10.21037/jtd.2018.02.85.
8. *Zhulkov MO, Sirota DA, Zykov IS, Sabetov AK, Agaeva KA, Makaev AG et al*. Results of a study of the effectiveness of direct coronary oxygen persufflation as a donor heart conditioning method. *Russian Journal of Transplantology and Artificial Organs*. 2022; 24 (3): 111–120. https://doi.org/10.15825/1995-1191-2022-3-111-120.
9. *Pinnelas R, Kobashigawa JA*. *Ex vivo* normothermic perfusion in heart transplantation: a review of the TransMedics® Organ Care System. *Future Cardiol*. 2022; 18 (1): 5–15. doi: 10.2217/fca-2021-0030.

10. *Slama A, Schillab L, Barta M, Benedek A, Mitterbauer A, Hoetzenecker K et al.* Standard donor lung procurement with normothermic *ex vivo* lung perfusion: A prospective randomized clinical trial. *J Heart Lung Transplant*. 2017; 36 (7): 744–753. doi: 10.1016/j.healun.2017.02.011.
11. *Jochmans I, O'Callaghan JM, Pirenne J, Ploeg RJ*. Hypothermic machine perfusion of kidneys retrieved from standard and high-risk donors. *Transpl Int*. 2015; 28 (6): 665–676. doi: 10.1111/tri.12530.
12. *Pettit SJ, Petrie MC*. Transplantation of Hearts Donated After Circulatory-Determined Death. *Circ Heart Fail*. 2019; 12 (4): e005991. doi: 10.1161/CIRCHEARTFAILURE.119.005991.
13. *Zhulkov MO, Fomichev AV, Alsov SA, Cleaver EN, Chernyavsky AM*. Current state of the problem and results of *ex vivo* perfusion of donor hearts. *Russian Journal of Transplantology and Artificial Organs*. 2019; 21 (4): 143–146. https://doi.org/10.15825/1995-1191-2019-4-143-146.
14. *Starling EH, Visscher MB*. The regulation of the energy output of the heart. *J Physiol*. 1927; 62 (3): 243–261. doi: 10.1113/jphysiol.1927.sp002355.
15. *Zimmer HG*. Modifications of the isolated frog heart preparation in Carl Ludwig's Leipzig Physiological Institute: relevance for cardiovascular research. *Can J Cardiol*. 2000; 16 (1): 61–69.
16. *Zimmer HG*. Who discovered the Frank-Starling mechanism? *News Physiol Sci*. 2002; 17: 181–184. doi: 10.1152/nips.01383.2002.
17. *Fye WB*. H. Newell Martin and the isolated heart preparation: the link between the frog and open heart surgery. *Circulation*. 1986; 73 (5): 857–864. doi: 10.1161/01.cir.73.5.857.
18. *Elzinga G*. Cross Talk Between Left and Right Heart: A Study on the Isolated Heart. Vrije Univ. te Amsterdam. 1972.
19. *Patterson SW, Starling EH*. The carbohydrate metabolism of the isolated heart lung preparation. *J Physiol*. 1913; 47 (1–2): 137–148. doi: 10.1113/jphysiol.1913.sp001617.
20. *Patterson SW, Starling EH*. On the mechanical factors which determine the output of the ventricles. *J Physiol*. 1914; 48 (5): 357–379. doi: 10.1113/jphysiol.1914.sp001669.
21. *Langendorff O*. Untersuchungen am überlebenden Säugethierherzen. *Arch für die gesamte Physiol des Menschen und der Tiere*. 1895; 61: 291–332.
22. *Neely JR, Liebermeister H, Battersby EJ, Morgan HE*. Effect of pressure development on oxygen consumption by isolated rat heart. *Am J Physiol*. 1967; 212 (4): 804–814. doi: 10.1152/ajplegacy.1967.212.4.804.
23. *Simaan J, Fawaz G*. The mechanical efficiency of the Starling heart-lung preparation. *Pflugers Arch*. 1968; 302 (2): 123–132. doi: 10.1007/BF00586865.
24. *Huisman PH, Schipperheyn JJ*. The isolated heart-lung preparation. The Hague: Martinus Nijhoff Medical Division, 1978. 36–36.
25. *Langer RM*. Vladimir P. Demikhov, a pioneer of organ transplantation. *Transplant Proc*. 2011; 43 (4): 1221–1222. doi: 10.1016/j.transproceed.2011.03.070.
26. *Kozlov IA, Krichevskiy LA*. Heart Transplantation in the USSR and Former Soviet Countries. *J Cardiothorac Vasc Anesth*. 2020; 34 (12): 3398–3408. doi: 10.1053/j.jvca.2019.12.001.
27. *Zhulkov MO, Zykov IS, Sirota DA, Agaeva HA, Sabetov AK, Poveschenko OV et al*. Long-Term Conditioning of a Donor Heart by Autoperfusion. *Journal of experimental and clinical surgery.* 2022; 15: 3: 214–220. doi: 10.18499/2070-478X-2022-15-3-214-220.

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AORTIC VALVE REPLACEMENT AFTER PREVIOUS TAVI

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Endovascular surgery for aortic valve defects has proven itself well in elderly patients with severe comorbidities competing with the underlying disease. However, the risk of dysfunction resulting from structural degeneration of bioprosthetic heart valve and prosthetic valve endocarditis remains high. Repeated surgeries are associated with complications, but open surgery is the only method of treatment in this group of patients. **Objective:** to describe a series of reinterventions for prosthetic valve dysfunction occurring after TAVI. **Material and methods.** From 2015 to 2022, at the Department of Emergency Surgery for Acquired Heart Diseases (Head, Professor R.M. Muratov), Bakulev Research Center for Cardiovascular Surgery, 6 reoperations were performed in patients who had previously undergone transcatheter aortic valve implantation (TAVI). The average age of patients at the time of TAVI and at the time of reoperation was 70.6 years (62–83) and 74.3 years (70–84), respectively. The EuroSCORE II predicted risk of mortality at the time of reintervention was 42.2% (21.7–87.6). The mean time to reoperation was 42 months. Indications for reoperation were early active prosthetic endocarditis (4 cases) and structural valve degeneration (2 cases). **Results.** At the hospital stage, 1 patient died of acute heart failure; the operation was performed for vital indications in conditions of extreme initial severity. In three patients, the early postoperative period was uneventful. One patient required intra-aortic balloon counterpulsation (IABP) due to heart failure, and 1 patient was implanted with permanent pacemaker. The average time of hospitalization was 14 days. Patients with active prosthetic endocarditis received a 6-week course of antibiotic therapy. The function of the implanted valves was satisfactory. **Conclusions.** Aortic valve replacement after previous TAVI is an emergency operation and represents the only way to treat valve dysfunction. Under active prosthetic endocarditis, timely surgery can save this patient cohort.

Keywords: valve replacement, infective endocarditis, TAVI.

INTRODUCTION

When choosing a surgical technique for aortic stenosis, it has been shown that open surgery is indicated in young patients, amidst infective endocarditis and the risk level according to STS and EuroSCORE II scales is below 4%. Whereas in the presence of multivessel coronary artery disease, atrioventricular valve pathology, aortic aneurysm, interventricular septal hypertrophy requiring myomectomy and the risk degree according to the same scales more than 4%, comorbid pathology, gross post-radiation changes in the mediastinal organs, risk of injury to functioning shunts during resternotomy may be a preference for transcatheter aortic valve replacement (TAVR, also called transcatheter aortic valve implantation, TAVI).

With the accumulation of experience in TAVR procedures, the disadvantages and contraindications have been identified. Absolute contraindications include the absence of specially trained cardiac surgical service, life expectancy <1 year, and low likelihood of improving the quality of life after TAVR due to severe concomitant pathology. Anatomical features such as narrow or wide aortic annulus (<18 mm or >29 mm) and left ventricular thrombus are also important. Unfavorable aortic root anatomy, asymmetric calcinosis with a high risk of coronary ostial obstruction, aortic atheromatosis with unstable plaques and a high risk of systemic embolism may also be contraindications.

The TAVI procedure was originally intended to be minimally invasive and to maximize safety for patients with high surgical risk. However, the incidence and extent of acute complications during valve implantation, such as coronary ostial obstruction, rupture of the aortic annulus, atrioventricular block, paraprosthetic fistulas, stroke, myocardial infarction and complications occurring at various times after surgery on the side of the implanted valve: secondary valve thrombosis, dysfunction due to compression and prosthetic endocarditis do not make this technique the gold standard when choosing the type of surgery for aortic valve (AV) stenosis.

MATERIALS AND METHODS

From 2015 to 2022, 6 patients were re-operated at the Department of Emergency Surgery of Acquired Heart Defects, Bakulev Research Center for Cardiovascular Surgery (led by Prof. R.M. Muratov) after earlier TAVR. The average age of patients at the time of TAVR was 70.6 years (62–83), at the time of reoperation was 74.3 years (70–84). The predicted EuroSCORE II mortality risk at the time of reintervention was 42.2%

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(21.7–87.6). Women/men were 4/2. The mean time to perform reoperation from the time of primary surgery was 42 months (8–144) (see Table). The indication for surgery in 4 cases was early active prosthetic valve endocarditis (PVE), in 2 cases structural degeneration of the valve.

The main symptoms in patients were dyspnea at minimal physical exertion, prolonged increase in body temperature up to 38.5 °C, loss of body weight, and severe weakness. All patients had severe heart failure (HF) with lower limb edema and enlarged liver. One patient was operated for vital indications due to progressive HF.

All patients were examined by standard methods. Cardiac ultrasound found that in patients with PVE, there were overlaps and vegetations on the prosthetic leaflets with formation of grade 2–3 mitral regurgitation. In patients with prosthetic degeneration, valve leaflets were limited in mobility, thickened and calcified with formation of transvalvular peak and mean gradient and significant regurgitation. In one case, in a patient with the CoreValve aortic valve, when it was difficult to confirm an infectious lesion using transesophageal echocardiogram (TEE) due to its structural features (high nitinol framework), 18F-FDG PET/CT was performed to confirm the diagnosis of PVE, which showed the presence of pathological hypermetabolism in the bioprosthetic aortic valve projection (see Fig. 1). To exclude malfor-

Table

Clinical characteristics of reoperated patients, n = 6

Patient / Age	TAVR system	Age at TAVR (years)	Age at re-operation (years)	Post-TAVR period (months)	ES 2 (%)	Concomitant pathology
1. 70 years	Edwards Sapien-23	65	70	60	19.9	MFA, COPD
2. 84 years	Core Valve-26	83	84	8	36.5	MFA, stage 4 CKD, diabetes, HBP
3. 73 years	Edwards Sapien-29	72	73	8	87.6	Stage 4 CKD. Benign prostatic hyperplasia
4. 75 years	MedLAB-27	73	75	24	24.8	Pulmonary fibrosis, HBP
5. 70 years	Boston Scientific Acurate neo-25	69	70	11	23.5	Mastectomy, radiotherapy
6. 74 years	CoreValve-23	62	74	144	38.1	Surgery artificial circulation and ECMO history

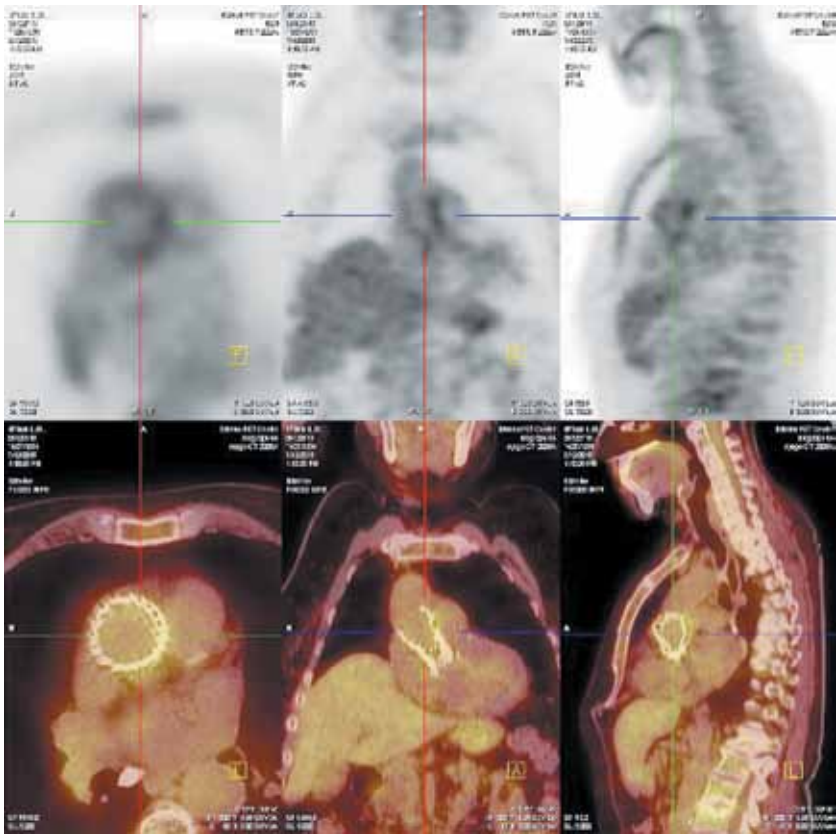

Fig. 1. 18F-FDG PET-CT imaging, pathological hypermetabolism in the bioprosthetic aortic valve projection

mation and cerebral mycotic aneurysm, brain MRI was performed in case of infective PVE.

CHARACTERISTICS OF SURGICAL INTERVENTIONS

All operations were performed under hypothermic cardiopulmonary bypass (28 °C). In five cases, the operation was performed through a full median sternotomy, in 1 case a J-ministernotomy along the 4th intercostal space was used. Myocardial protection in all patients was performed by administration of 2 liters of Custodiol solution. The average cardiopulmonary bypass (CPB) time was 197.5 minutes and aortic clamping time was 141.7 minutes.

Patient 1. Diagnosis: postoperative Edwards Sapien-23 TAVR by transapical access condition. Bioprosthetic aortic valve stenosis and failure on the background of structural degeneration (Fig. 2). Stage IIb heart failure (HF). New York Heart Association (NYHA) functional class IV. Operation: AV replacement with BioLAB-20 bioprosthetic valve. CPB lasted for 116 minutes. Aortic clamping lasted for 76 minutes. The postoperative period was uneventful; the patient was discharged on day 8.

Patient 2. Diagnosis: postoperative CoreValve 26 TAVR condition, stenting of the left anterior descending artery (LADA). Early prosthetic AV endocarditis, active phase. Infective mitral valve (MV) endocarditis with grade 2 regurgitation. Stent restenosis in the LADA. Stage IIb HF. NYHA functional class IV. Operation: AV replacement with Karboniks-22 mechanical prosthetic valve, MV replacement with Karboniks-28 mechanical prosthetic valve, coronary artery bypass grafting (CABG-LADA) (see Fig. 3). CPB lasted for 227 minutes. Aortic clamping lasted for 165 minutes. The postoperative period was uneventful; the patient was discharged on day 18.

Patient 3. Diagnosis: postoperative Edwards Sapien-29 TAVR condition. Early prosthetic AV endocarditis,

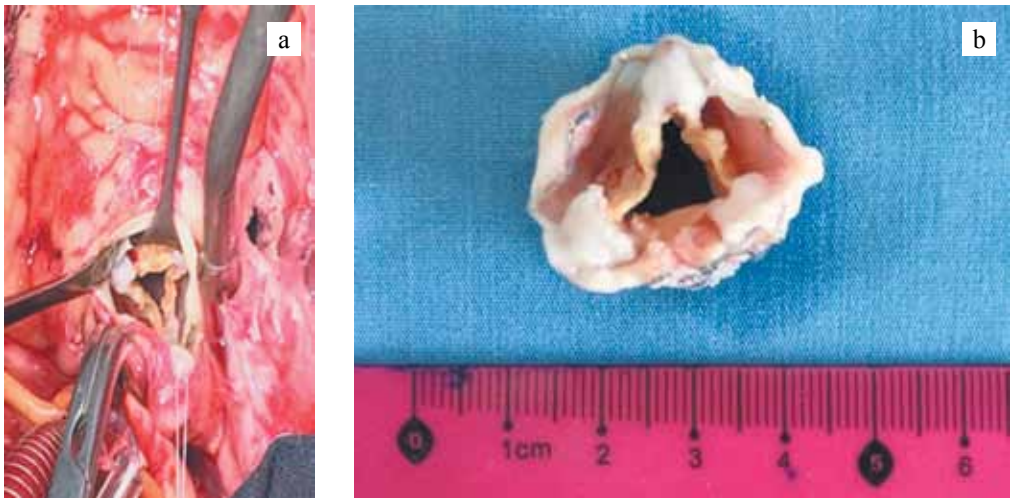

Fig. 2. Structural degeneration of the Edwards Sapien-23 valve: a, intraoperative photo; b, explanted Edwards Sapien-23 valve

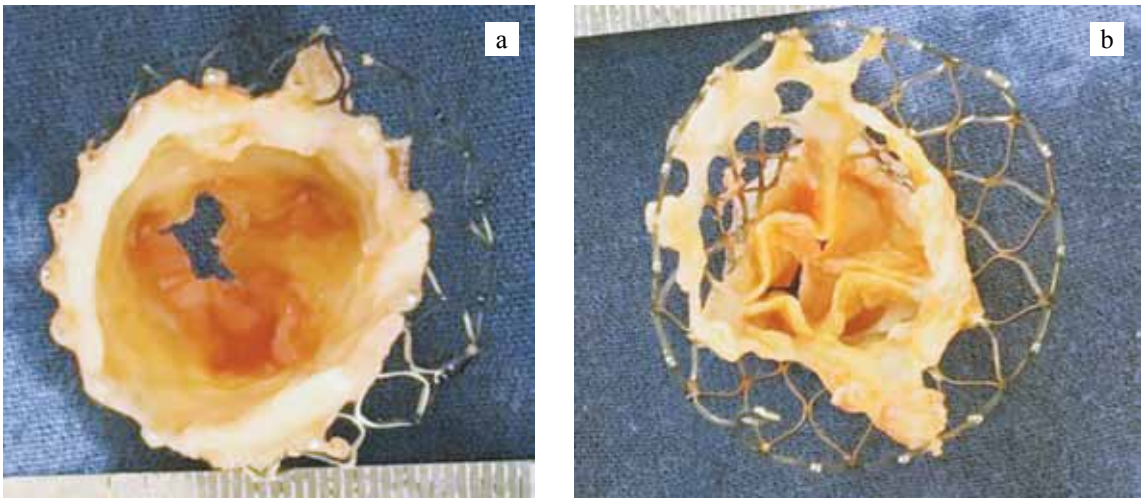

Fig. 3. Prosthetic valve endocarditis affecting the CoreValve 26 stent (vegetation on xenopericardial flaps): a, ventricular surface of the valve; b, aortic surface of the valve

active phase. Grade 3 mitral and tricuspid regurgitation. High pulmonary hypertension. Ascites. Artificial ventilation. Stage IIb HF. NYHA functional class IV. Operation: AV replacement with Karboniks-26, MV repair on a polytetrafluoroethylene strip, tricuspid valve repair according to DeVega procedure (see Fig. 4). CPB lasted for 204 minutes. Aortic clamping lasted for 125 minutes. The patient died from progressive HF in the early postoperative period.

Patient 4. Diagnosis: postoperative TAVI MedLab-KT 27 condition. Early aortic prosthetic valve endocarditis. Aortic regurgitation. Stage IIa HF. NYHA functional class III. Operation: AV replacement with BioLAB-22 bioprosthetic valve from a mini-sternotomy (Fig. 5). CPB lasted for 151 minutes. Aortic clamping lasted for 85 minutes. The postoperative period was uneventful; the patient was discharged on day 9.

Patient 5. Diagnosis: postoperative Boston Scientific Acurate neo-25 condition. Early prosthetic AV endocarditis, active phase. Aortic regurgitation (see Fig. 6). Stage IIa HF. NYHA functional class IV. Operation: aortic root replacement with devitalized allograft, implantation of a dual-chamber pacemaker. CPB lasted for 194 minutes. Aortic clamping lasted for 140 minutes. The postoperative period was uneventful; the patient was discharged on day 16.

Patient 6. Diagnosis: postoperative Mitroflow aortic valve bioprosthesis, AV replacement using CoreValve 23 TAVR technique under ECMO to prevent postoperative complication in the patient with critical hemodynamic disturbance (intraoperative ventricular tachycardia). Structural degeneration of the valve by calcinosis (see Fig. 7). Stage IIa HF. NYHA functional class II. Operation: AV replacement with Karboniks-22 prosthetic valve.

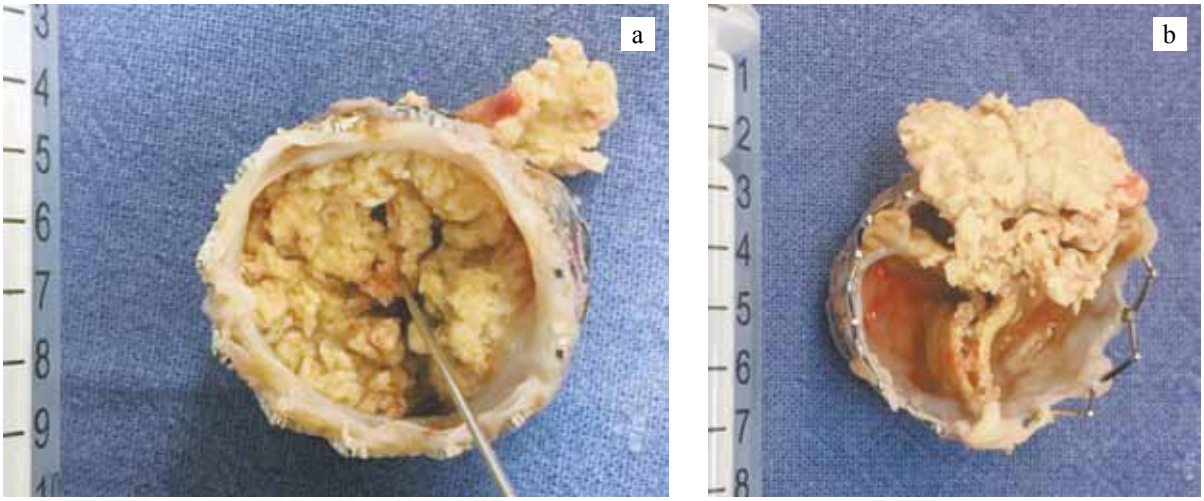

Fig. 4. Prosthetic valve endocarditis affecting the Edwards Sapien-29 valve stent: a, ventricular surface of the valve; b, aortic surface of the valve

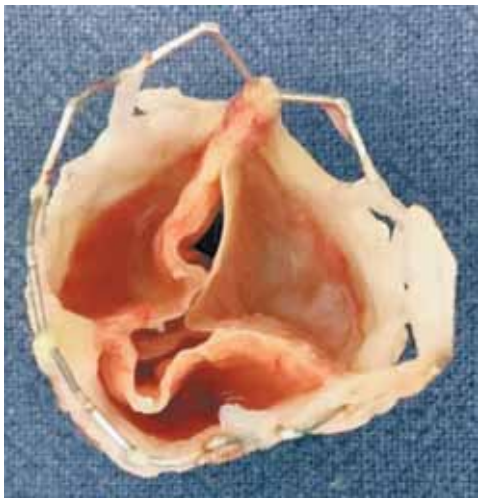

Fig. 5. Explanted MedLAB KT 27 valve stent (flat vegetations on PTFE flaps)

RESULTS

At the hospital stage, 1 patient died of acute HF. Patient #3 with decompensated HF was taken for surgery on vital indications with multi-organ failure. EUROScore II was 87%. To prevent and replenish blood loss during all operations, the Cell-Saver device was used. Blood loss by drains on the first day averaged 500 mL (350–750). Mechanical ventilation lasted for an average of 23 [13 : 682] hours. Prolonged ventilation was performed due to neurological deficit in patient #3 and development of HF. The patient stayed at the ICU for 4.5 (1.3–30) days.

In three patients, the early postoperative period was uneventful. The average time in hospital was 14 days. Patients with active PVE underwent a 6-week course of antibiotic therapy. At the moment of discharge, 3 patients

had sinus rhythm, 1 patient had permanent atrial fibrillation, and 1 patient had a permanent pacemaker. The function of implanted prosthetic valves was satisfactory.

DISCUSSION

The development and implementation of alternative techniques (TAVI, balloon valvuloplasty) for the treatment of AV stenosis in high surgical risk and inoperable patients (STS 7–11%, EuroSCORE II 18 29%) have shown safety and stable outcomes at various times after surgery [1]. However, expansion of indications for transcatheter procedures is often an example of commercial advantage without in-depth discussion of each specific clinical case. The decision to perform them should be made and discussed by a group of physicians from different specialties [2].

A retrospective MedPAR (Medicare Provider Analysis and Review) analysis summarizing data from 2009 to 2015 in patients with isolated AV stenosis showed an increase in the number of AV interventions by 14.4% per year (from 22,076 in 2009 to 49,362 in 2015). When comparing the number of surgeries performed (traditional AV replacement and TAVI), there is an increasing trend for catheter-based procedures. By the end of the study, such procedures accounted for 46% of all AV interventions. The authors also emphasized a downward trend in in-hospital 30- and 90-day mortality, which were 2.69%, 4.46%, 6.66%, respectively. However, 90-day mortality in the TAVI group remains high at 8.37% and the incidence of infective endocarditis ranges from 2.4% to 2.7% [3].

Infective endocarditis after TAVI is a life-threatening complication with high in-hospital and 1-year mortality. Early diagnosis is of paramount importance in order to initiate appropriate treatment to avoid negative clinical outcomes. According to the TAVI International Registry, the incidence of PVE ranges from 0.3 to 1.2% of patients per year. The authors identified the use of

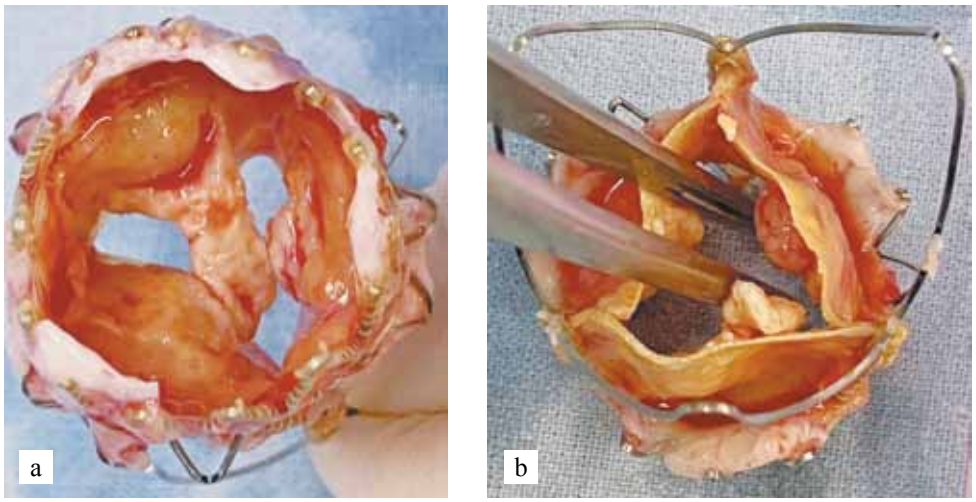

Fig. 6. Prosthetic valve endocarditis affecting the Boston Scientific Acurate neo-25 valve stent: a, ventricular surface of the valve; b, aortic surface of the valve

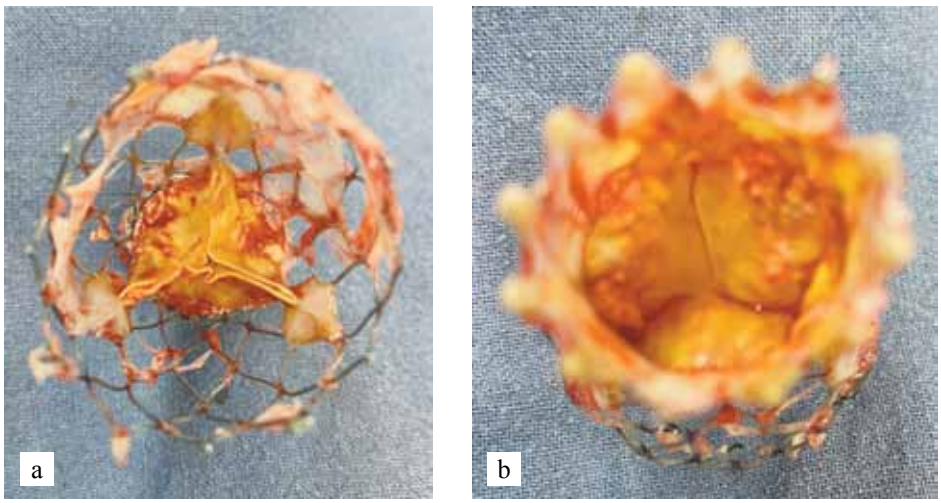

Fig. 7. Structural degeneration of the CoreValve 23 valve stent: a, ventricular surface of the valve; b, aortic surface of the valve

self-expandable stent valves and intubation during surgery as independent predictors. Transfemoral access was performed in 76% of cases. The authors also noted high in-hospital mortality (47%), both at the time of TAVI and at reoperation. These results are associated with the initial severity and age of the patients [4]. Our material describes four clinical cases of early PVE after transcatheter AV implantation, and it should be said that despite certain difficulties in interpreting the diagnosis of "prosthetic valve endocarditis" especially in the case of implanted CoreValve valve, the use of active surgical tactics with the use of conventional surgery, in combination with antibiotic therapy, has shown to be feasible and effective in this severe category of recurrent cases.

A pooled analysis of 61 randomized controlled trials analyzing 8,969 patients, which covered the period from 2012 to 2020, found no significant difference in the endpoint, deaths from cardiovascular causes. A positive effect was observed in surrogate endpoints such as bleeding, postoperative atrial fibrillation impairing quality of life, renal failure, strokes, and length of hospital stay. Meta-analysis demonstrated no significant difference in mortality in the early and mid-term postoperative mortality. Moreover, by year 5, mortality in the TAVI group had increased by 15% (OR 1.11 95% CI 1.01–1.23, p = 0.04, I^2 – 0%; n = 3761) [5–8]. The early postoperative mortality may be due to the occurrence of events such as strokes (2-year study, OR 0.88, 95% CI 0.67 to 1.16, p = 0.37, I2 48%; 6 studies, n = 6,453 patients) [5–9], bleeding (reported 64% reduction in major bleeding in favor of TAVI, this trend persists up to year 5 where the value is 20%). By year 5, the observed effect had shifted toward a favorable prognosis for surgical AV replacement.

Regarding surgical aspects, this technique has several technical limitations [10–12], resulting in an increased number of re-interventions (including valve-to-valve) and hospitalizations (see Fig. 8). A PORTICO study [13] showed that the absence of anticoagulant therapy, valve-to-valve procedure, use of a 23-mm transcatheter valve, and high BMI were independent predictors of hemodynamic valve deterioration. This hypothesis is supported by the fact that the problem associated with prosthetic leaflet thrombosis was resolved by long-term warfarin use.

Infective endocarditis, as the most dangerous complication at up to 30 days, shows a significant decrease in the TAVI group; further follow-up shows that this effect has no differences and by year 5 the weighted mean reaches an OR value of 1.34 (95% CI 0.87–2.05, P = 0.18, I2 0%; 4 studies, n = 3,761) [1, 14–16], and in relative terms, the number of infective endocarditis cases increases by 134%.

However, such data should be alarming and must be treated with some caution. Pooled analysis of 6 cohort studies demonstrated that individuals with early PVE were younger (73.5 ± 4.2 vs 79.9 ± 3.24, P < 0.001), identified the most significant risk factors as sex OR 1.24 (95% CI 1.15 to 1.33), intubation OR 2.99 (95% CI 2.73 to 3.28), and chronic kidney disease (OR 5.19, 95% CI 4.16 to 6.47). The median time to development of infective endocarditis ranged from 1 month to 9.4 months [17–20]. The analysis demonstrated that only 22.3% were re-operated, the rest (77.7%) were treated conservatively. Overall mortality for the pooled cohort was 38.3%. Mortality in the surgical and antibiotic treatment groups were 16.7% and 37.4% (P < 0.05) [21].

CONCLUSION

In order to determine the surgical tactics for degenerative aortic valve defects, in the context of modern realities with the development and wide accessibility of endovascular aids, it is necessary to adhere to the point of view of pragmatism, where the end and means must be justified. In today's era of saturated information flow and patient awareness, indications for the method of choice must be clearly defined. TAVI should be performed in advanced centers for possible elimination of the developed complication. The decision to choose a method should be based on such indicators as durability of the prosthetic valve and life expectancy, rather than the classical approach based on modern risk stratification scales and anatomical features.

Fatal complications such as PVE in this patient cohort are associated with an enormous risk of mortality. However, only a combination of surgical treatment and conservative therapy can provide a predictable outcome and the possibility of cure.

The authors declare no conflict of interest.

REFERENCES

1. *Baumgartner H, Falk V, Bax JJ, De Bonis M, Hamm C, Holm PJ et al.* 2017 ESC/EACTS Guidelines for the management of valvular heart disease. *Eur Heart J*. 2017 Sep 21; 38 (36): 2739–2791. https://doi.org/10.1093/eurheartj/ehx391.
2. *Nishimura RA, Otto CM, Bonow RO, Carabello BA, Erwin JP 3rd, Guyton RA et al*. 2014 AHA/ACC Guideline for the Management of Patients With Valvular Heart Disease. *J Am Coll Cardiol*. 2014 Jun 10; 63 (22): e57–e185; doi: 10.1016/j.jacc.2014.02.536.
3. *Culler SD, Cohen DJ, Brown PP, Kugelmass AD, Reynolds MR, Ambrose K et al.* Trends in Aortic Valve Replacement Procedures Between 2009 and 2015: Has Transcatheter Aortic Valve Replacement Made a Difference? *Ann Thorac Surg*. 2018 Apr; 105 (4): 1137–1143. doi: https://doi.org/10.1016/j.athoracsur.2017.10.057.
4. *Amat-Santos IJ, Messika-Zeitoun D, Eltchaninoff H, Kapadia S, Lerakis S, Cheema AN et al.* Infective endocarditis after transcatheter aortic valve implantation: results from a large multicenter registry. *Circulation*. 2015 May 5; 131 (18): 1566–1574. doi: 10.1161/CIRCULATIONAHA.114.014089. PMID: 25753535.

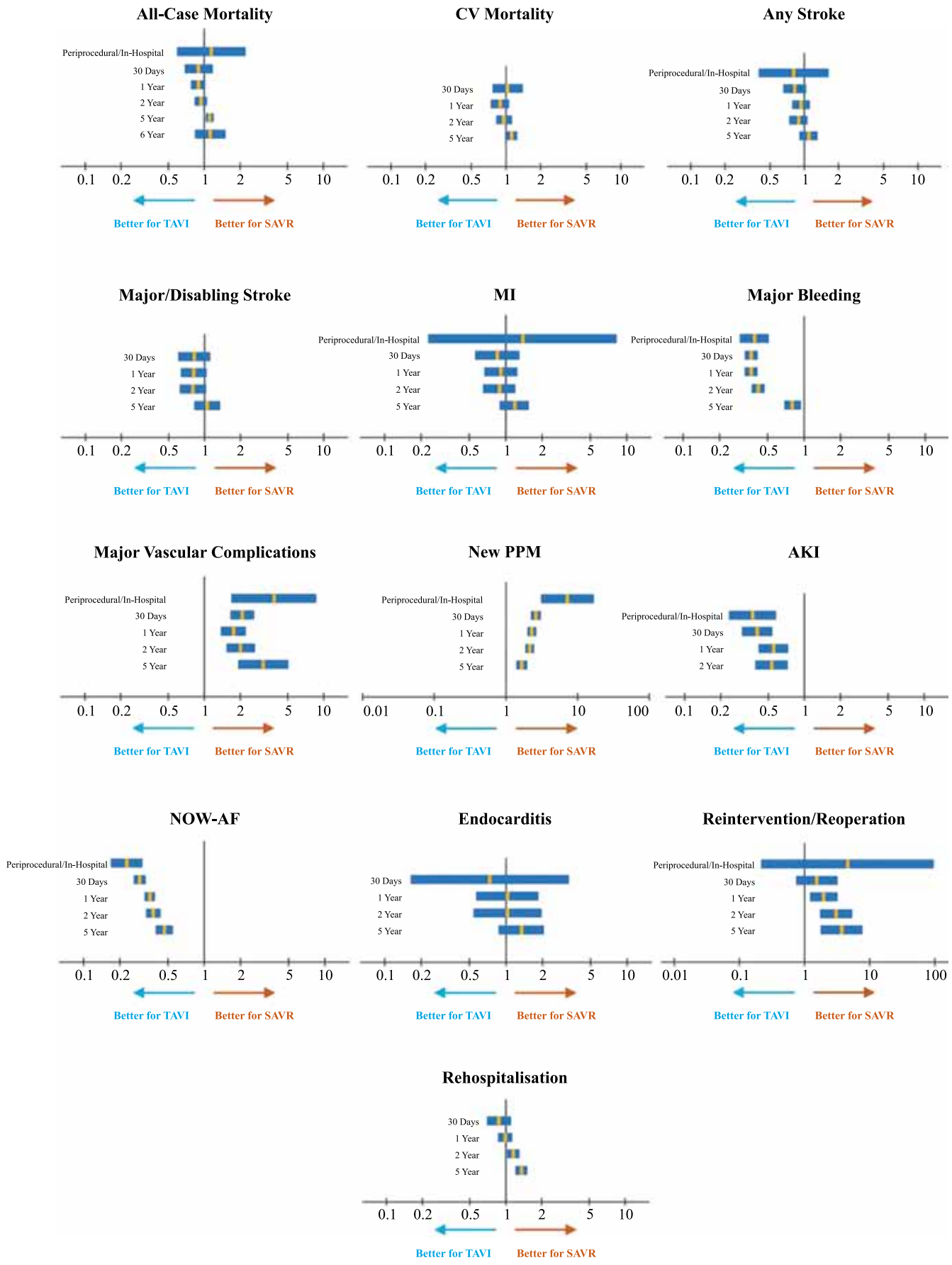

Fig. 8. Data pooled from randomized controlled trials (RCT) results [22]

5. *Leon MB, Smith CR, Mack MJ, Makkar RR, Svensson LG, Kodali SK et al*. Transcatheter or Surgical Aortic-Valve Replacement in Intermediate-Risk Patients. *N Engl J Med*. 2016 Apr 28; 374 (17): 1609–1620. https://doi.org/10.1056/NEJMoa1514616.
6. *Adams DH, Popma JJ, Reardon MJ, Yakubov SJ, Coselli JS, Deeb GM et al*. Transcatheter aortic-valve replacement with a self-expanding prosthesis. *N Engl J Med*. 2014 May 8; 370 (19): 1790–1798. https://doi.org/10.1056/NEJMoa1400590.
7. *Thyregod HG, Steinbrüchel DA, Ihlemann N, Nissen H, Kjeldsen BJ, Petursson P et al*. Transcatheter Versus Surgical Aortic Valve Replacement in Patients With Severe Aortic Valve Stenosis: 1-Year Results From the All-Comers NOTION Randomized Clinical Trial. *J Am Coll Cardiol*. 2015 May 26; 65 (20): 2184–2194. https://doi.org/10.1016/j.jacc.2015.03.014.
8. *Smith CR, Leon MB, Mack MJ, Miller DC, Moses JW, Svensson LG et al*. Transcatheter versus surgical aortic-valve replacement in high-risk patients. *N Engl J Med*. 2011 Jun 9; 364 (23): 2187–2198. https://doi.org/10.1056/NEJMoa1103510.
9. *Reardon MJ, Van Mieghem NM, Popma JJ, Kleiman NS, Søndergaard L, Mumtaz M et al*. Surgical or transcatheter aortic-valve replacement in intermediate-risk patients. *N Engl J Med*. 2017 Apr 6; 376 (14): 1321–1331. doi: 10.1056/NEJMoa1700456.
10. *de Buhr W, Pfeifer S, Slotta-Huspenina J, Wintermantel E, Lutter G, Goetz WA*. Impairment of pericardial leaflet structure from balloon-expanded valved stents. *J Thorac Cardiovasc Surg*. 2012 Jun; 143 (6): 1417–1421. https://doi.org/10.1016/j.jtcvs.2011.11.001.
11. *Amahzoune B, Bruneval P, Allam B, Lafont A, Fabiani JN, Zegdi R*. Traumatic leaflet injury during the use of percutaneous valves: a comparative study of balloon-and self-expandable valved stents. *Eur J Cardiothorac Surg*. 2013 Mar; 43 (3): 488–493. https://doi.org/10.1093/ejcts/ezs359.
12. *Alavi SH, Groves EM, Kheradvar A*. The effects of transcatheter valve crimping on pericardial leaflets. *Ann Thorac Surg*. Apr; 97 (4): 1260–1266. doi: 10.1016/j.athoracsur.2013.11.009.
13. *Del Trigo M, Muñoz-Garcia AJ, Wijeysundera HC, Nombela-Franco L, Cheema AN, Gutierrez E et al*. Incidence, Timing, and Predictors of Valve Hemodynamic Deterioration After Transcatheter Aortic Valve Replacement: Multicenter Registry. *J Am Coll Cardiol*. 2016 Feb 16; 67 (6): 644–655. https://doi.org/10.1016/j.jacc.2015.10.097.
14. *Bonow RO, Brown AS, Gillam LD, Kapadia SR, Kavinsky CJ, Lindman BR et al*. ACC/AATS/AHA/ASE/EACTS/HVS/SCA/SCAI/SCCT/SCMR/STS 2017 appropriate use criteria for the treatment of patients with severe aortic stenosis: a report of the American College of cardiology appropriate use criteria Task force, American association for thoracic surgery, American heart association, American Society of echocardiography, European association for Cardio-Thoracic surgery, heart valve Society, society of cardiovascular Anesthesiologists, Society for cardiovascular angiography and interventions, society of cardiovascular computed tomography, Society for cardiovascular magnetic resonance, and society of thoracic surgeons. *J Am Soc Echocardiogr*. 18 Feb; 31 (2): 117–147. doi: 10.1016/j.echo.2017.10.020.
15. *Barili F, Freemantle N, Pilozzi Casado A, Rinaldi M, Folliguet T, Musumeci F et al*. Mortality in trials on transcatheter aortic valve implantation versus surgical aortic valve replacement: a pooled meta-analysis of Kaplan–Meier-derived individual patient data. *Eur J Cardiothorac Surg*. 2020 Aug 1; 58 (2): 221–229. https://doi.org/10.1093/ejcts/ezaa087.
16. *Wang D, Huang L, Zhang Y, Cheng Z, Zhang X, Ren P et al*. Transcatheter aortic valve implantation versus surgical aortic valve replacement for treatment of severe aortic stenosis: comparison of results from randomized controlled trials and real-world data. *Braz J Cardiovasc Surg*. 2020 Jun 1; 35 (3): 346–367. doi: 10.21470/1678-9741-2019-0288. PMID: 32549107; PMCID: PMC7299574.
17. *Weintrob AC, Roediger MP, Barber M, Summers A, Fieberg AM, Dunn J et al*. Natural history of colonization with gram-negative multidrug-resistant organisms among hospitalized patients. *Infect Control Hosp Epidemiol*. 2010 Apr; 31 (4): 330–337. doi: 10.1086/651304. PMID: 20175687. https://www.ncbi.nlm.nih.gov/pubmed/20175687.
18. *Gallouche M, Barone-Rochette G, Pavese P, Bertrand B, Vanzetto G, Bouvaist H et al*. Incidence and prevention of infective endocarditis and bacteraemia after transcatheter aortic valve implantation in a French university hospital: a retrospective study. *J Hosp Infect*. 2018 May; 99 (1): 94–97. doi: 10.1016/j.jhin.2017.11.013. PMID: 29191610. https://www.ncbi.nlm.nih.gov/pubmed/29191610.
19. *Martínez-Sellés M, Bouza E, Díez-Villanueva P, Valerio M, Fariñas MC, Muñoz-García AJ et al*. Incidence and clinical impact of infective endocarditis after transcatheter aortic valve implantation. *EuroIntervention*. 2016 Feb; 11 (10): 1180–1187. doi: 10.4244/EIJY15M02_05. PMID: 25671426. https://www.ncbi.nlm.nih.gov/pubmed/25671426.
20. *Regueiro A, Linke A, Latib A, Ihlemann N, Urena M, Walther T et al*. Association between transcatheter aortic valve replacement and subsequent infective endocarditis and in-hospital death. *JAMA*. 2016 Sep 13; 316 (10): 1083–1092. doi: 10.1001/jama.2016.12347. PMID: 27623462. https://www.ncbi.nlm.nih.gov/pubmed/27623462.
21. *Tinica G, Tarus A, Enache M, Artene B, Rotaru I, Bacusca A, Burlacu A*. Infective endocarditis after TAVI: a meta-analysis and systematic review of epidemiology, risk factors and clinical consequences. *Rev Cardiovasc Med*. 2020 Jun 30; 21 (2): 263–274. doi: 10.31083/j.rcm.2020.02.68. PMID: 32706214.
22. *Swift SL, Puehler T, Misso K, Lang SH, Forbes C, Kleijnen J et al*. Transcatheter aortic valve implantation versus surgical aortic valve replacement in patients with severe aortic stenosis: a systematic review and meta-analysis. *BMJ Open*. 2021 Dec 6; 11 (12): e054222. https://doi.org/10.1136/bmjopen-2021-054222.

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