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RECENT HISTORY OF TRANSPLANTOLOGY: 35 YEARS OF HEART TRANSPLANTATION AND 25 YEARS OF LIVING RELATED LIVER TRANSPLANTATION IN THE RUSSIAN FEDERATION

Глубокоуважаемые коллеги!

Открывая любую книгу по трансплантологии, мы обязательно найдем в ней упоминание о глубоких многовековых корнях этого современного инновационного направления медицины со ссылкой на древнеегипетские папирусы или чудо Святых Космы и Дамиана, пришивающих белокожему реципиенту ногу мавра. И хотя мне очень импонирует тезис о том, что идея пересадки органов существует столько же, сколько существует сама медицина, все же реальная история клинической трансплантации вершилась при жизни нынешнего поколения врачей и пациентов, можно сказать, не только на наших глазах, но и в значительной мере – нашими руками. В 2022 году мы отмечаем две значимые даты в истории отечественной трансплантологии – 35 лет успешной трансплантации сердца и 25 лет родственной трансплантации печени детям.

Три с половиной десятилетия назад академик В.И. Шумаков положил начало новой эпохе лечения терминальной сердечной недостаточности в нашей стране, выполнив первую успешную трансплантацию сердца и подарив погибающей двадцатидевятилетней пациентке еще почти десять лет полноценной жизни.

К началу 2022 года количество выполненных в нашей стране трансплантаций сердца

Dear colleagues,

Whenever you open any book on transplantology, you are bound to find a mention of the deep centuries-old roots of this modern, innovative area of medicine with reference to the ancient Egyptian papyri or Saints Cosmas and Damian who miraculously transplanted the black leg of an Ethiopian man onto a white Roman church official to replace his cancerous limb. Although I am very impressed by the thesis that the idea of organ transplantation has existed as long as medicine itself has, the real history of clinical transplantation was actually made in the lifetime of the current generation of doctors and patients, not only before our very eyes but to a large extent, by our hands as well. In 2022, we are celebrating two significant dates in the history of Russian transplantology – 35 years of successful heart transplantation and 25 years of pediatric living related liver transplantation.

Three and a half decades ago, world-renowned Russian surgeon and transplantologist professor Valery Shumakov initiated a new era in the treatment of end-stage heart failure in our country; he performed the first successful heart transplant in our country to a 29-year-old dying patient who lived after for about 10 years.

приблизилось к 2200 операциям, причем почти две трети из них выполнено в НМИЦ ТИО им. академика В.И. Шумакова. В числе прочих семнадцати клинических центров, работающих по программе трансплантации сердца, наиболее активно работа велась в Национальном медицинском исследовательском центре им. В.А. Алмазова (г. Санкт-Петербург), Национальном медицинском исследовательском центре им. ак. Е.Н. Мешалкина (г. Новосибирск) и Краевой клинической больнице № 1 им. проф. С.В. Очаповского (г. Краснодар).

Четверть века назад нами было положено начало новому направлению – трансплантации фрагментов печени детям от живых (родственных) доноров, что позволило излечивать детей с врожденными холестатическими заболеваниями, приводящими к фатальной печеночной недостаточности. Трансплантацию фрагмента печени от родственного донора мы выполняем детям с предельно малой массой тела, начиная с первых месяцев их жизни. Теперь, имея опыт более тысячи таких трансплантаций, мы можем констатировать, что в нашей стране полностью удовлетворяется потребность в указанном виде медицинской помощи.

21–23 сентября 2022 года – даты проведения в Москве XI Всероссийского съезда трансплантологов с международным участием. Центральными темами пленарных заседаний, мастер-классов, дискуссий, научных докладов являются проблемы – возникающие и решаемые – в различных областях отечественной и зарубежной трансплантологии, клеточных технологий и регенеративной медицины.

Уверен, что возможность живого общения, ведение высокопрофессионального диалога, анализ и обсуждение научно-практических вопросов, обмен опытом и уникальными клиническими наблюдениями во время съезда трансплантологов будут полезны для выработки конструктивных решений и послужат стимулом к появлению инновационных идей.

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

By the beginning of 2022, the number of heart transplants performed in our country was close to 2,200; almost two-thirds of them were done at Shumakov National Medical Research Center of Transplantology and Artificial Organs in Moscow. Among the other 17 heart transplant centers, the most active so far were Almazov National Medical Research Centre in St. Petersburg, Meshalkin National Medical Research Center in Novosibirsk and Ochapovsky Regional Clinical Hospital No. 1 in Krasnodar.

A quarter of a century ago, we laid the foundation for a new direction – pediatric living related liver transplantation. This has allowed us to cure children with congenital cholestatic diseases that lead to fatal liver failure. We perform liver transplant from a living related donor in children with extremely low body weight, starting from the first months of birth. Now, having performed over a thousand such transplants, we can categorically state that the need for this type of medical care is being fully met in our country.

The 11th All-Russian Congress of Transplantologists is scheduled to hold on September 21–23, 2022 in Moscow, featuring international participants. The central topics of plenary sessions, master classes, discussions, and scientific reports are the problems of cell technologies and regenerative medicine that are emerging and being addressed in various fields of national and foreign transplantology.

I am confident that the Congress which will be featuring face-to-face contact, highly professional dialogue, analysis and discussion of scientific and practical issues, exchange of experience and unique clinical observations will facilitate constructive solutions and stimulate innovative ideas.

Sincerely,

S.V. Gautier
Editor-in-Chief,
Member, Russian Academy of Sciences

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ORGAN DONATION AND TRANSPLANTATION IN THE RUSSIAN FEDERATION IN 2021

14th 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 2021. **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 transplantation centers. **Results.** Based on data retrieved from the 2021 Registry, 45 kidney, 29 liver and 17 heart transplantation programs were existing in the Russian Federation as of the year 2021. The kidney transplant waiting list in 2021 included about 10.5% of the 60,000 patients receiving dialysis. Organ donation activity in 2021 was 4.5 per million population, with a 78.4% multi-organ procurement rate and an average of 3.0 organs procured from one effective donor. In 2021, there were 9.5 kidney transplants per million population, 4.2 liver transplants per million population and 2.0 heart transplants per million population. Same year, the number of transplant surgeries performed in the Russian Federation increased by 18.3% compared to the year 2020, reaching the level of 2019. In Moscow, organ donation activity was 23.7 per million population, that of 2019. In 2021, the city of Moscow and the Moscow region accounted for 12 functioning organ transplant centers, performing 57.7% of all kidney transplants and 70.5% of all extrarenal transplants in the country. The number of organ recipients in the Russian Federation has exceeded 140 per million population. **Conclusion.** In 2021, donor activity and volume of transplant care in Russian regions recovered. This was after the decline in 2020 that resulted from the new coronavirus disease (COVID-19) pandemic. In addition, 7 new transplant programs were established. Further development of regional organ donation and transplantation programs, improvement in their efficiency, increase in the activity of transplant centers and development of inter-regional collaboration are expected in the Russian Federation in 2022.

Keywords: organ donation, kidney, liver, heart, lung transplantation, transplant center, waiting list, registry, COVID-19, 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 organ transplant commission created by the Russian Ministry of Health and the Russian Transplant Society. Previous reports have been published in 2009–2021 [1–12].

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 human donor 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 from the Registry contain not only statistical data for the reporting period, but also systematic analysis of the data with an assessment of the current state of transplantation care in the Russian Federation, trends and prospects for further development in this healthcare sector.

Since 2019, the Registry has also been used for monitoring the implementation of the departmental target program "*Organ Donation and Transplantation in the Russian Federation"*, approved via executive order

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No. 365 of the Russian Ministry of Health, dated June 4, 2019.

Data for the Registry is collected via questionnaires administered to appropriate officials at all transplantation 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 permanent 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 AND WAITING LISTS

In the Russian Federation, there are transplant centers in 35 federal subjects with a total population of 103.4 million people (see Fig. 1).

In 2021, kidney transplantation (KT) was performed in 45 centers, liver transplantation (LiT) in 29, heart transplantation (HT) in 17, pancreas transplantation (PTx) in 3, lung transplantation (LnT) in 3, and small bowel transplantation in 1.

In 2021, various transplant interventions were performed in 57 medical institutions. Of these, 19 were federal institutions, including 12 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 38 are institutions run by federal subjects of the Russian Federation.

Eight medical institutions that were hosting transplant centers did not perform organ transplants in 2021 due to their repurposing for treatment of COVID-19 patients.

In 2021, there were 6,313 potential recipients on the KT waiting list in the Russian Federation, i.e., 10.5% of the total number of patients on hemodialysis and peritoneal dialysis (about 60,000 according to unpublished data from the Registry of the Russian Dialysis Society). Of these, 1,567 were waitlisted in 2021 for the first time. There were 2,272 potential recipients on the LiT waiting list in 2021; 886 of them were included in the list for the first time in 2021. In 2021, there were 736 potential recipients included in the HT waiting list; 326 of them were included in the list for the first time in 2021. Between 2012 and 2021, as the number of organ transplants increased in the Russian Federation, the number of patients waitlisted for KT almost doubled, the LiT waiting list increased 4.7 times, while HT waitlist increased 1.8 times [4–12].

In 2021, 2,318 organ transplants (15.9 per million population) were performed in Russia – 271 were pediatric organ transplants. See Tables 1 and 2.

The number of organ transplants in the Russian Federation increased by 18.3% (+358) compared to 2020. The rate of increase in transplant activity in the Russian Federation in 2021 was higher by 43.2% than was envisaged in 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.

From 124 (in January) to 230 (in November) organ transplants were performed monthly – about 200 on average. See Fig. 2.

In 2021, 69 to 138 KT, 37 to 69 LiT and 15 to 30 HT were performed per month in the Russian Federation.

Based on data obtained from the Federal Registry for High-Tech Medical Care, 2,052 (88.5%) organ transplant surgeries were performed in 2021, using funds from the compulsory medical insurance system that were allocated for provision of high-tech medical care for organ transplant. There were 1,842 (94.0%) of such surgeries in 2020. See Fig. 3. Another 266 (11.5%) organ transplants were performed using funds from the federal subjects of the Russian Federation and the federal budget.

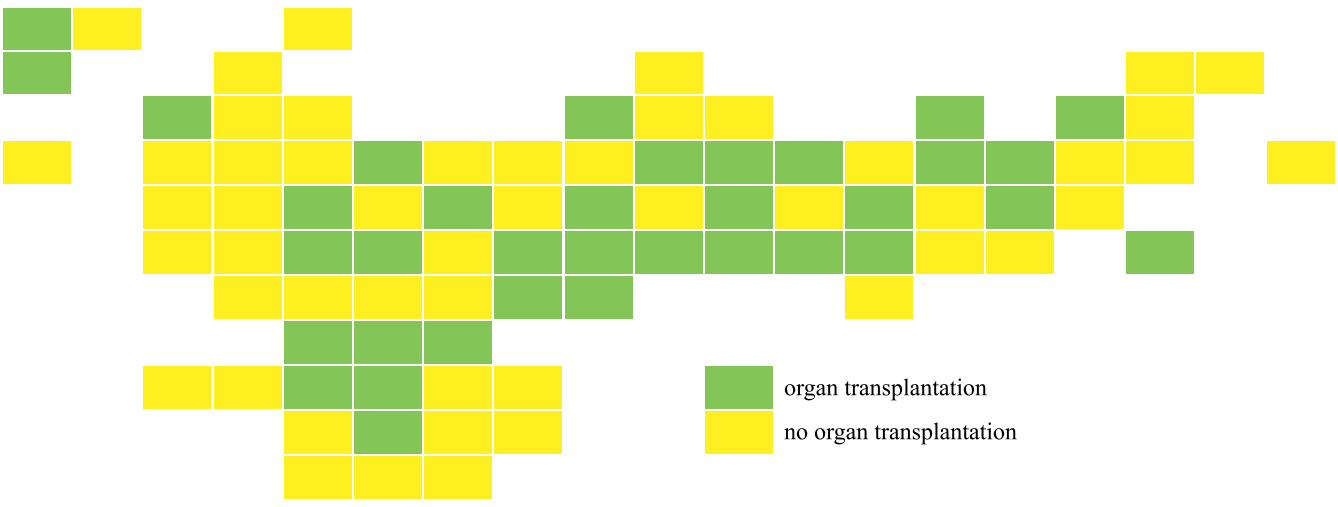

Fig. 1. Geographic distribution of organ transplant centers in Russia in 2021

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 2.6-fold. Meanwhile, the proportion of organ transplants performed using these funds has increased by 30.3%.

The financial costs per unit of high-tech medical care for transplantation in 2021 were as follows:

– 991,870 rubles for kidney, pancreas, kidney-pancreas, small bowel, lung transplant;
– 1,257,557 rubles for heart-liver transplant;
– 1,797,532 rubles for heart-lung transplant.

(Resolution No. 2299 of the Government of the Russian Federation, dated December 28, 2020).

ORGAN DONATION

In 2021 donor programs were carried out in 33 federal subjects of the Russian Federation.

In Perm Krai, the Republic of Sakha (Yakutia), and the Republic of Buryatia, only living related KT were performed.

In 2021, new donor programs were launched in 3 federal subjects of the Russian Federation. There are:

– Republic of Buryatia, living related kidney donation,
– Primorsky Krai, deceased organ donation,
– Ivanovo Oblast, deceased organ donation.

There were a total of 652 effective deceased organ donors (4.5 per million population) in 2021. See Table 3.

Effective deceased organ donors in the Russian Federation grew by 15.6% (+88) compared to 2020.

Table 1

Organ donation and transplantation in the Russian Federation in 2021

Indicator	Number (abs.)	Indicator per million population*
Organ donation		
Total number of organ donors	1,016	6.9
Deceased donors	652	4.5
Living (related) donors	364	2.5
Organ transplantation		
Total number of organs transplanted	2318	15.9
share of pediatric transplants	271	–
Kidney	1,384	9.5
from deceased donors	1,183	–
from living-related donors	201	–
share of pediatric transplants	122	–
Liver	618	4.2
from deceased donors	455	–
from living-related donors	163	–
share of pediatric transplants	134	–
Heart	290	2.0
share of pediatric transplants	15	–
Pancreas	10	0.1
Lungs	13	0.1
share of pediatric transplants	0	–
Heart-lung	2	–
share of pediatric transplants	0	–
Small bowel	1	–
share of pediatric transplants	0	–

* Population of the Russian Federation in 2021: 146.2 million people (www.gks.ru).

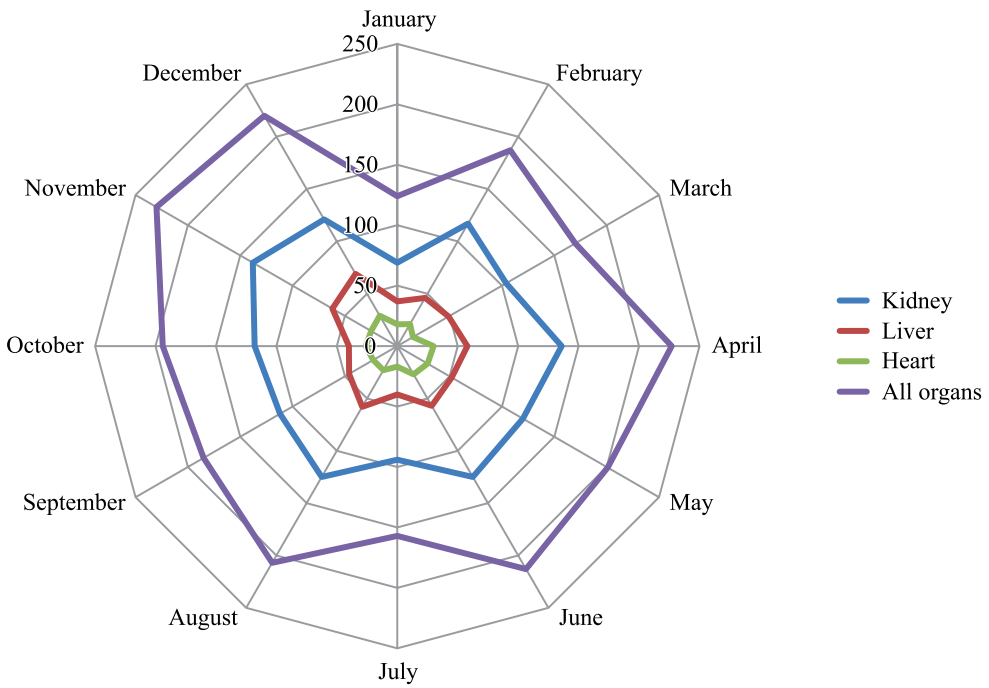

Fig. 2. Organ transplantation by month in 2021

Table 2

Transplant activity in the Russian Federation in 2021

S/N	Transplant center, region, federal district	**Total**	**Kidney (total)**	Kidney (cadaveric)	Kidney (living related)	**Liver (total)**	Liver (cadaveric)	Liver (living related)	**Heart**	**Pancreas**	**Lungs**	**Heart-lungs**	**Small bowel**
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**	**684**	**283**	185	98	**177**	76	101	**211**	**2**	**9**	**2**	**0**
1.2.	Branch of the Shumakov National Medical Research Center of Transplantology and Artificial Organs, **Volzhsky, Southern Federal District**	**32**	**28**	12	16	**4**	4	0	**0**	**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**	**51**	**51**	39	12	**0**	0	0	**0**	**0**	**0**	**0**	**0**
3	Russian Children's Clinical Hospital, **Moscow, Central Federal District**	**40**	**40**	38	2	**0**	0	0	**0**	**0**	**0**	**0**	**0**
4	Petrovsky National Research Centre of Surgery, **Moscow, Central Federal District**	**23**	**14**	12	2	**8**	0	8	**0**	**1**	**0**	**0**	**0**
5	Burnazyan Federal Medical and Biophysical Center, **Moscow, Central Federal District**	**54**	**13**	11	2	**41**	14	27	**0**	**0**	**0**	**0**	**0**
6	Bakulev Scientific Center of Cardiovascular Surgery, **Moscow, Central Federal District**	**3**	**0**	0	0	**0**	0	0	**3**	**0**	**0**	**0**	**0**
7	National Medical Research Center for Children's Health, **Moscow, Central Federal District**	**15**	**15**	3	12	**0**	0	0	**0**	**0**	**0**	**0**	**0**
8	Botkin City Clinical Hospital, **Moscow, Central Federal District**	**127**	**87**	87	0	**40**	40	0	**0**	**0**	**0**	**0**	**0**
9	Sklifosovsky Research Institute of Emergency Care, **Moscow, Central Federal District**	**369**	**231**	231	0	**122**	121	1	**5**	**7**	**3**	**0**	**1**
10	Loginov Moscow Clinical Research and Practical Center, **Moscow, Central Federal District**	**6**	**0**	0	0	**6**	6	0	**0**	**0**	**0**	**0**	**0**
11	Vladimirsky Moscow Regional Research Clinical Institute, **Moscow Oblast, Central Federal District**	**59**	**40**	40	0	**19**	19	0	**0**	**0**	**0**	**0**	**0**
12	Federal Clinical Center for High Medical Technologies under the Federal Biomedical Agency (119), **Moscow Oblast, Central Federal District**	**27**	**25**	19	6	**0**	0	0	**2**	**0**	**0**	**0**	**0**
13	St. Joasaphus Belgorod Regional Clinical Hospital, **Belgorod, Central Federal District**	**5**	**4**	4	0	**1**	1	0	**0**	**0**	**0**	**0**	**0**
14	Voronezh Regional Clinical Hospital No. 1, **Voronezh, Central Federal District**	**4**	**4**	4	0	**0**	0	0	**0**	**0**	**0**	**0**	**0**
15	Tula Regional Clinical Hospital, **Tula, Central Federal District**	**5**	**5**	3	2	**0**	0	0	**0**	**0**	**0**	**0**	**0**
16	Ryazan Regional Clinical Hospital, **Ryazan, Central Federal District**	**15**	**11**	11	0	**4**	4	0	**0**	**0**	**0**	**0**	**0**
17	Ivanovo Regional Clinical Hospital, **Ivanovo Oblast, Central Federal District**	**1**	**1**	1	0	**0**	0	0	**0**	**0**	**0**	**0**	**0**

Continuation table 2

1	2	3	4	5	6	7	8	9	10	11	12	13	14
18	Stavropol Regional Clinical Hospital, **Stavropol, North Caucasian Federal District**	**10**	**6**	6	0	**4**	4	0	**0**	**0**	**0**	**0**	**0**
19	Ochapovsky Regional Clinical Hospital No. 1, **Krasnodar, Southern Federal District**	**45**	**27**	27	0	**10**	10	0	**8**	**0**	**0**	**0**	**0**
20	Volzhsky Regional Urological Center, **Volzhsky, Southern Federal District**	**20**	**20**	7	13	**0**	0	0	**0**	**0**	**0**	**0**	**0**
21	Rostov Regional Clinical Hospital, **Rostov-on-Don, Southern Federal District**	**56**	**35**	35	0	**14**	13	1	**7**	**0**	**0**	**0**	**0**
22	Russian Research Center of Radiology and Surgical Technologies, **St. Petersburg, Northwestern Federal District**	**12**	**0**	0	0	**12**	12	0	**0**	**0**	**0**	**0**	**0**
23	Almazov National Medical Research Centre, **St. Petersburg, Northwestern Federal District**	**22**	**0**	0	0	**0**	0	0	**22**	**0**	**0**	**0**	**0**
24	Pavlov First St. Petersburg State Medical University, **St. Petersburg, Northwestern Federal District**	**26**	**19**	16	3	**6**	6	0	**0**	**0**	**1**	**0**	**0**
25	St. Petersburg Research Institute of Emergency Medicine, **St. Petersburg, Northwestern Federal District**	**22**	**20**	20	0	**2**	2	0	**0**	**0**	**0**	**0**	**0**
26	City Mariinskaya Hospital, **St. Petersburg, Northwestern Federal District**	**10**	**10**	10	0	**0**	0	0	**0**	**0**	**0**	**0**	**0**
27	Kirov Military Medical Academy, **St. Petersburg, Northwestern Federal District**	**10**	**0**	0	0	**10**	10	0	**0**	**0**	**0**	**0**	**0**
28	Leningrad Regional Clinical Hospital, **St. Petersburg, Northwestern Federal District**	**22**	**22**	22	0	**0**	0	0	**0**	**0**	**0**	**0**	**0**
29	Volosevich First City Clinical Hospital, **Arkhangelsk, Northwestern Federal District**	**2**	**2**	1	1	**0**	0	0	**0**	**0**	**0**	**0**	0
30	Meshalkin National Medical Research Center, **Novosibirsk, Siberian Federal District**	**8**	**0**	0	0	**0**	0	0	**8**	**0**	**0**	**0**	**0**
31	State Novosibirsk Regional Clinical Hospital, **Novosibirsk, Siberian Federal District**	**72**	**25**	19	6	**47**	29	18	**0**	**0**	**0**	**0**	**0**
32	Research Institute for Complex Issues of Cardiovascular Diseases, **Kemerovo, Siberian Federal District**	**3**	**0**	0	0	**0**	0	0	**3**	**0**	**0**	**0**	**0**
33	Belyaev Kemerovo Regional Clinical Hospital, **Kemerovo, Siberian Federal District**	**51**	**51**	49	2	**0**	0	0	**0**	**0**	**0**	**0**	**0**
34	Podgorbunsky Regional Clinical Emergency Hospital, **Kemerovo, Siberian Federal District**	**4**	**0**	0	0	**4**	4	0	**0**	**0**	**0**	**0**	**0**
35	Irkutsk Regional Clinical Hospital, **Irkutsk, Siberian Federal District**	**18**	**11**	11	0	**7**	7	0	**0**	**0**	**0**	**0**	**0**
36	Regional Clinical Hospital, Altai Krai (Barnaul), **Siberian Federal District**	**17**	**16**	16	0	**1**	1	0	**0**	**0**	**0**	**0**	**0**
37	Federal Center for Cardiovascular Surgery, **Krasnoyarsk, Siberian Federal District**	**1**	**0**	0	0	**0**	0	0	**1**	**0**	**0**	**0**	**0**
38	Federal Siberian Research and Clinical Center, **Krasnoyarsk, Siberian Federal District**	**21**	**17**	17	0	**4**	4	0	**0**	**0**	**0**	**0**	**0**
39	Regional Clinical Hospital, **Krasnoyarsk, Siberian Federal District**	**27**	**16**	16	0	**8**	8	0	**3**	**0**	**0**	**0**	**0**
40	Sverdlovsk Regional Clinical Hospital No. 1, **Yekaterinburg, Ural Federal District**	**41**	**27**	27	0	**11**	11	0	**3**	**0**	**0**	**0**	**0**
41	Chelyabinsk Regional Clinical Hospital, **Chelyabinsk, Ural Federal District**	**10**	**6**	6	0	**2**	2	0	**2**	**0**	**0**	**0**	**0**
42	Regional Clinical Hospital No. 1, **Tyumen, Ural Federal District**	**15**	**12**	12	0	**1**	1	0	**2**	**0**	**0**	**0**	**0**

End of table 2

1	2	3	4	5	6	7	8	9	10	11	12	13	14
43	District Clinical Hospital, **Khanty-Mansiysk, Ural Federal District**	**9**	**6**	4	2	**1**	1	0	**2**	**0**	**0**	**0**	**0**
44	Samara State Medical University, **Samara, Volga Federal District**	**47**	**47**	46	1	**0**	0	0	**0**	**0**	**0**	**0**	**0**
45	Saratov State Medical University, **Saratov, Volga Federal District**	**8**	**8**	0	8	**0**	0	0	**0**	**0**	**0**	**0**	**0**
46	Regional Clinical Hospital, **Saratov, Volga Federal District**	**4**	**4**	4	0	**0**	0	0	**0**	**0**	**0**	**0**	**0**
47	Volga Regional Medical Center, **Nizhny Novgorod, Volga Federal District**	**28**	**14**	11	3	**14**	7	7	**0**	**0**	**0**	**0**	**0**
48	Republican Clinical Hospital, **Kazan, Volga Federal District**	**80**	**52**	49	3	**28**	28	0	**0**	**0**	**0**	**0**	**0**
49	Interregional Clinical Diagnostic Center, **Kazan, Volga Federal District**	**3**	**0**	0	0	**0**	0	0	**3**	**0**	**0**	**0**	**0**
50	Republican Clinical Hospital, **Ufa, Volga Federal District**	**50**	**40**	40	0	**10**	10	0	**0**	**0**	**0**	**0**	**0**
51	Republican Cardiology Clinic, **Ufa, Volga Federal District**	**5**	**0**	0	0	**0**	0	0	**5**	**0**	**0**	**0**	**0**
52	Perm Regional Clinical Hospital, **Perm, Volga Federal District**	**3**	**3**	0	3	**0**	0	0	**0**	**0**	**0**	**0**	**0**
53	City Clinical Hospital for Emergency Medical Care No. 1, **Orenburg, Volga Federal District**	**6**	**6**	6	0	**0**	0	0	**0**	**0**	**0**	**0**	**0**
54	Republican Hospital No. 1 – National Center of Medicine, **Yakutsk, Far Eastern Federal District**	**2**	**2**	0	2	**0**	0	0	**0**	**0**	**0**	**0**	**0**
55	Semashko Republican Clinical Hospital, **Ulan-Ude, Far Eastern Federal District**	**2**	**2**	0	2	**0**	0	0	**0**	**0**	**0**	**0**	**0**
56	Primorsky Regional Clinical Hospital No. 1, **Vladivostok, Far Eastern Federal District**	**6**	**6**	6	0	**0**	0	0	**0**	**0**	**0**	**0**	**0**
Total		**2318**	**1384**	**1183**	**201**	**618**	**455**	**163**	**290**	**10**	**13**	**2**	**1**

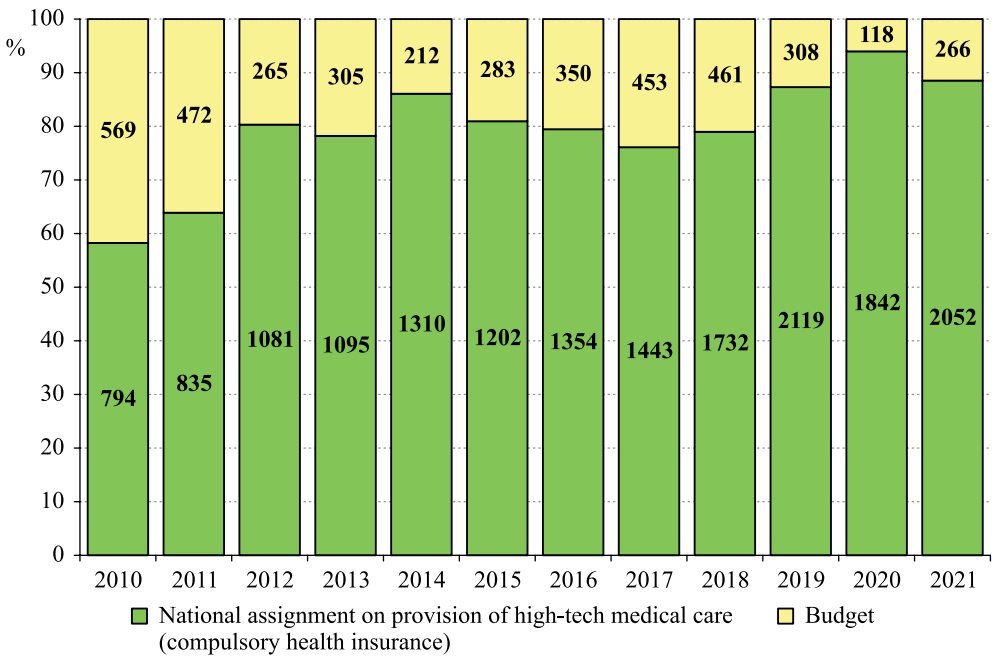

Fig. 3. Funding for organ transplants in the Russian Federation in 2010–2021

Table 3

Indicators associated with organ donation activity in the regions of the Russian Federation in 2021

S/N	Region	Organ Donation Coordinating Center (region)	Population (million)	Number of donor bases	Effective donors (absolute, per million population)		including brain-dead donors (absolute, %)		including multi-organ donors (absolute, %)		Total number of organs harvested	including harvested kidneys	Organ-to-donor ratio	Percentage of harvested kidneys
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	Moscow	Moscow Coordinating Center for Organ Donation, Moscow (Botkin City Clinical Hospital)	12.6	23	298	23.7	290	97.3	250	83.9	957	545	3.2	91.4
2	Moscow Oblast	Vladimirsky Moscow Regional Research Clinical Institute, Moscow	7.7	13	36	4.7	35	97.2	32	88.9	128	68	3.6	94.4
3	Belgorod Oblast	St. Joasaphus Belgorod Regional Clinical Hospital, Belgorod	1.5	1	2	1.3	2	100.0	1	50.0	5	4	2.5	100.0
4	Voronezh Oblast	Voronezh Regional Clinical Hospital No. 1, Voronezh	2.3	3	3	1.3	3	100.0	1	33.3	8	6	2.7	100.0
5	Tula Oblast	Tula Regional Clinical Hospital, Tula	1.4	1	4	2.9	4	100.0	4	100.0	14	7	3.5	87.5
6	Ryazan Oblast	Ryazan Regional Clinical Hospital, Ryazan	1.1	1	11	10.0	11	100.0	7	63.6	33	21	3.0	95.5
7	Ivanovo Oblast	Ivanovo Regional Clinical Hospital, Ivanovo	1.0	1	1	1.0	1	100.0	1	100.0	4	2	4.0	100.0
8	Krasnodar Krai	Ochapovsky Regional Clinical Hospital No. 1, Krasnodar	5.7	2	13	2.3	13	100.0	12	92.3	44	26	3.4	100.0
9	Volgograd Oblast	Branch of Shumakov National Medical Research Center of Transplantology and Artificial Organs, Volzhsky, Southern Federal District	2.5	2	10	4.0	10	100.0	4	40.0	26	19	2.6	95.0
10	Rostov Oblast	Rostov Regional Clinical Hospital, Rostov-on-Don	4.2	1	21	5.0	21	100.0	21	100.0	63	42	3.0	100.0
11	Stavropol Krai	Stavropol Regional Clinical Hospital, Stavropol, North Caucasian Federal District	2.8	1	5	1.8	5	100.0	4	80.0	14	10	2.8	100.0
12	St. Petersburg	Center for Organ and Tissue Donation, St. Petersburg (St. Petersburg Research Institute of Emergency Medicine)	5.4	5	25	4.6	25	100.0	25	100.0	84	44	3.4	88.0
13	Leningrad Oblast	Leningrad Regional Clinical Hospital, St. Petersburg	1.9	1	12	6.3	12	100.0	9	75.0	35	22	2.9	91.7
14	Arkhangelsk Oblast	Volosevich First City Clinical Hospital, Arkhangelsk, Northwestern Federal District	1.1	1	1	0.9	1	100.0	0	0.0	4	2	4.0	100.0
15	Novosibirsk Oblast	State Novosibirsk Regional Clinical Hospital, Novosibirsk	2.8	4	15	5.4	14	93.3	13	86.7	39	21	2.6	70.0

End of table 3

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
16	Kemerovo Oblast	Belyaev Kemerovo Regional Clinical Hospital, Kemerovo	2.6	15	28	10.8	18	64.3	15	53.6	74	52	2.6	92.9
17	Irkutsk Oblast	Irkutsk Regional Clinical Hospital, Irkutsk	2.4	1	8	3.3	8	100.0	7	87.5	18	11	2.3	68.8
18	Altai Krai	Regional Clinical Hospital, Barnaul	2.3	1	7	3.0	7	100.0	6	85.7	20	14	2.9	100.0
19	Krasnoyarsk Krai	Krasnoyarsk Clinical Hospital, Krasnoyarsk	2.9	12	12	4.1	12	100.0	10	83.3	32	18	2.7	75.0
20	Sverdlovsk Oblast	Sverdlovsk Regional Clinical Hospital No. 1, Yekaterinburg Chelyabinsk Regional Clinical Hospital, Chelyabinsk	4.3	2	14	3.3	14	100.0	12	85.7	41	27	2.9	96.4
21	Chelyabinsk Oblast	Chelyabinsk Regional Clinical Hospital, Chelyabinsk	3.4	1	3	0.9	3	100.0	2	66.7	10	6	3.3	100.0
22	Tyumen Oblast	Regional Clinical Hospital No. 1, Tyumen	1.5	3	8	5.3	8	100.0	4	50.0	21	14	2.6	87.5
23	Khanty-Mansi Autonomous Okrug – Yugra	District Clinical Hospital, Khanty-Mansiysk	1.7	1	2	1.2	2	100.0	2	100.0	7	4	3.5	100.0
24	Samara Oblast	Samara State Medical University, Samara	3.1	4	24	7.7	18	75.0	3	12.5	48	47	2.0	97.9
25	Saratov Oblast	Regional Clinical Hospital, Saratov	2.4	1	6	2.5	6	100.0	4	66.7	16	12	2.7	100.0
26	Nizhny Novgorod Oblast	Volga Regional Medical Center, Nizhny Novgorod	3.2	3	7	2.2	7	100.0	6	85.7	18	11	2.6	78.6
27	Republic of Tatarstan	Republican Clinical Hospital, Kazan	3.9	2	35	9.0	35	100.0	32	91.4	84	51	2.4	72.9
28	Republic of Bashkortostan	Republican Clinical Hospital, Ufa	4.0	6	21	5.3	21	100.0	12	57.1	55	40	2.6	95.2
29	Orenburg Oblast	City Clinical Hospital for Emergency Medical Care No. 1, Orenburg	1.9	1	4	2.1	4	100.0	4	100.0	12	8	3.0	100.0
30	Primorsky Krai	Primorsky Regional Clinical Hospital No. 1, Vladivostok	1.9	1	3	1.6	3	100.0	0	0.0	6	6	2.0	100.0
31	Departmental program of the Federal Biomedical Agency of the Russian Federation	Burnazyan Federal Medical and Biophysical Center, Moscow	–	1	3	–	3	100.0	3	100.0	13	6	4.3	100.0
32	Departmental Program of the Federal Biomedical Agency of the Russian Federation	Federal Siberian Research and Clinical Center, Krasnoyarsk	–	4	10	–	10	100.0	5	50.0	23	17	2.3	85.0
		Total	**146.2**	**119**	**652**	**4.5**	**626**	**96.0**	**511**	**78.4**	**1956**	**1183**	**3.0**	**90.7**

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 2021, the proportion of effective deceased organ donors >60 years of age was 16.1% (see Fig. 4). Male donors were 65.2%, females were 34.8%.

Donor activity per population of the regions implementing donor programs (95.5 million) amounted to 6.8 per million population (see Tables 4 and 5).

Moscow posted the highest donor activity at the European level – 23.7 per million population (20.9 in 2020). In two more federal subjects of the Russian Federation, Kemerovo Oblast and Ryazan Oblast, donor activity exceeded 10.0 per million population. In Ryazan Oblast, the level of donor activity increased almost twofold from 5.5 to 10.8 per million population; similarly, donor activity significantly increased in the Republic of Tatarstan from 5.4 to 9.0 effective deceased donors per million population.

In 2021, Irkutsk Oblast and Stavropol Krai witnessed a drop in donor activity – from 6.7 to 3.3 and from 4.6 to 1.8 effective postmortem donors per million population, respectively.

Moscow and Moscow Oblast accounted for 51.2% (334) of effective donors in 2021.

There were 626 effective brain-dead donors – 96.0% of the total pool of effective donors (see Fig. 5).

All donor programs use a protocol for determining human death based on brain death diagnosis. In 25 federal subjects of the Russian Federation, organ donor programs worked only with brain-dead donors.

There were 511 multi-organ procurements in 2021, accounting for 78.4% of the total number

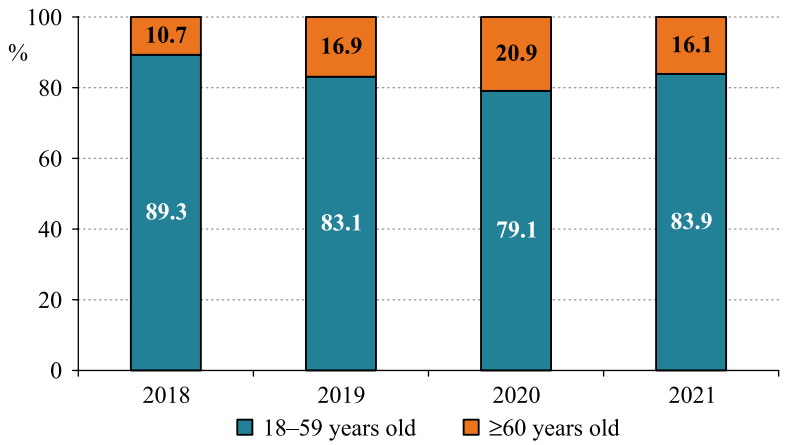

Fig 4. structure of effective organ donors in 2018–2021

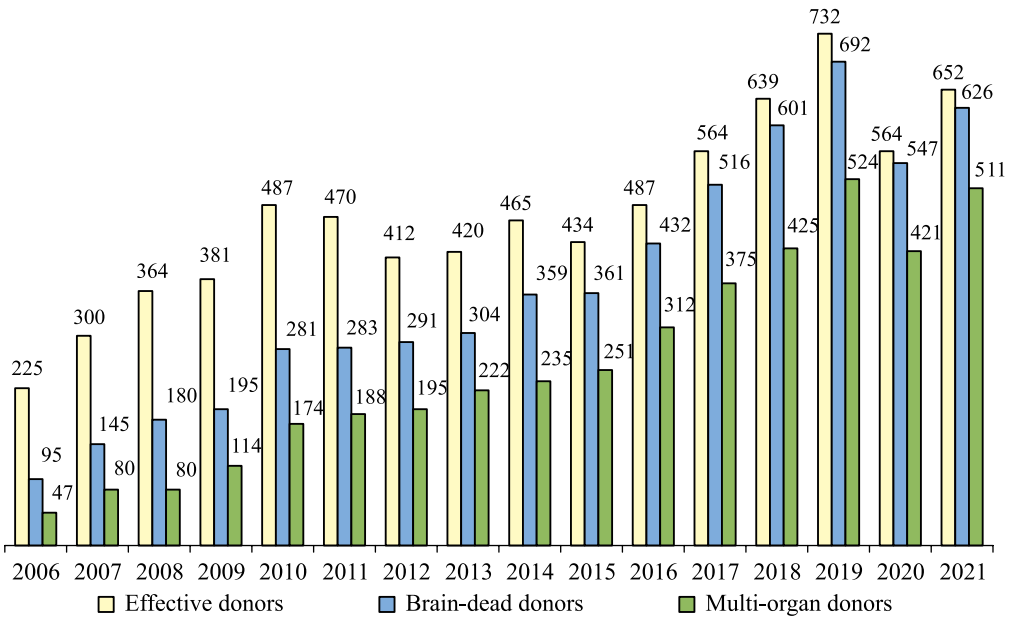

Fig. 5. Structure of effective organ donors in the Russian Federation in 2006–2021

of procurements (652). Compared to the year 2020, the number of multi-organ procurements increased by 21.4% (+90). In 18 donor programs, multiple organs were procured from >70% of patients, and in 6 programs, multi-organ procurement was done in all (100%) the patients. The 6 programs are Tula Oblast, Ivanovo Oblast, Rostov Oblast, St. Petersburg, Khanty-Mansi Autonomous Okrug – Yugra, and Orenburg Oblast.

Moscow and Moscow Oblast accounted for 282 multi-organ donors (55.2% of the total number of multi-organ donors) in the country in 2021.

The average number of organs procured from one donor in 2021 was 3.0 (2.9 in 2020). The highest number of organ procurements, as before, came from federal subjects that performed extrarenal organ transplantation and/or at federal subjects where there was interregional coordination: Moscow Oblast (3.6); Tula Oblast (3.5), Khanty-Mansi Autonomous Okrug – Yugra (3.5), St. Petersburg (3.4), Moscow (3.2), Ryazan Oblast (3.0), and Rostov Oblast (3.0).

In 2021, donor kidney utilization exceeded 90.0%, reaching 90.7%. In 22 regions, utilization was within the optimal 90–100% range, in 3 regions it was between 80% and 90%, and in 5 programs it was <80%.

Table 4

Rating of regions by donor activity in 2021

Federal Subject of the Russian Federation (Region)	Population in 2020 (million)	Number of effective donors (per million population)		Ranking		Change in ranking
		2021	2020	2021	2020	
Moscow	12.6	23.7	20.9	1	1	–
Kemerovo Oblast	2.7	10.8	10.0	2	2	–
Ryazan Oblast	1.1	10.0	5.5	3	6	+3
Republic of Tatarstan	3.9	9.0	5.4	4	8	+4
Samara Oblast	3.2	7.7	7.5	5	3	–2
Leningrad Oblast	1.8	6.3	6.1	6	5	–1
Novosibirsk Oblast	2.8	5.4	5.4	7	7	–
Republic of Bashkortostan	4.1	5.3	4.4	8	11	+3
Tyumen Oblast	1.5	5.3	3.3	9	16	+7
Rostov Oblast	4.2	5.0	4.3	10	12	+2
Moscow Oblast	7.7	4.7	2.8	11	17	+6
St. Petersburg	5.4	4.6	4.6	12	9	–3
Krasnoyarsk Krai*	2.9	4.1	3.4	13	15	–2
Volgograd Oblast	2.5	4.0	4.0	14	13	–1
Irkutsk Oblast	2.4	3.3	6.7	15	4	–11
Sverdlovsk Oblast	4.3	3.3	1.4	16	23	+7
Altai Krai	2.3	3.0	3.9	17	14	–3
Tula Oblast	1.4	2.9	2.0	18	19	+1
Saratov Oblast	2.4	2.5	0.0	19	29	+10
Krasnodar Krai	5.6	2.3	2.3	20	18	–2
Nizhny Novgorod Oblast	3.2	2.2	1.6	21	22	+1
Orenburg Oblast	1.9	2.1	0.5	22	28	+6
Stavropol Krai	2.8	1.8	4.6	23	10	–13
Primorsky Krai	1.9	1.6	0.0	24	–	+9
Belgorod Oblast	1.5	1.3	1.3	25	24	–1
Voronezh Oblast	2.3	1.3	1.7	26	21	–5
KhMAO - Yugra	1.7	1.2	1.8	27	20	–7
Ivanovo Oblast	1.0	1.0	0.0	28	–	+9
Arhangelsk Oblast	1.1	0.9	0.9	29	26	–3
Chelyabinsk Oblast	3.5	0.9	0.9	30	27	–3
Omsk Oblast	1.9	0.0	1.1	31	25	–6
The Republic of Sakha (Yakutia)	1.0	0.0	0.0	32	30	–2
Russia (85 federal subjects of the Russian Federation)	146.2	4.5	3.9		–	

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

Table 5

Deceased organ donors in 2006–2021

S/N	Region	2006	2007		2008		2009		2010		2011		2012		2013		2014		2015		2016		2017		2018		2019		2020		2021	
		Number of effective donors	Number of effective donors	Change over the year (abs.)	Number of effective donors	Change over the year (abs.)	Number of effective donors	Change over the year (abs.)	Number of effective donors	Change over the year (abs.)	Number of effective donors	Change over the year (abs.)	Number of effective donors	Change over the year (abs.)	Number of effective donors	Change over the year (abs.)	Number of effective donors	Change over the year (abs.)	Number of effective donors	Change over the year (abs.)	Number of effective donors	Change over the year (abs.)	Number of effective donors	Change over the year (abs.)	Number of effective donors	Change over the year (abs.)	Number of effective donors	Change over the year (abs.)	Number of effective donors	Change over the year (abs.)	Number of effective donors	Change over the year (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
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
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
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
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
5	Tula Oblast																										2	+2	3	+1	4	+1
6	Ryazan Oblast																								2	+2	13	+11	6	–7	11	+5
7	Ivanovo Oblast																														1	+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
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
10	Rostov Oblast																		1	+1	7	+6	13	+6	19	+6	21	+2	18	–3	21	+3
11	Stavropol Krai																								2	+2	3	+1	13	+10	5	–8
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
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
14	Arkhangelsk Oblast																								5	+5	5	0	1	–4	1	0
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
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
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
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

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
19	Altai Krai														3	+3	5	+2	4	–1	4	0	8	+4	8	0	8	0	9	+1	7	–2
20	Krasnoyarsk Krai																3	+3	6	+3	6	0	27	+9	16	Note	13	–3	10	–3	12	+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
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
23	Tyumen Oblast																						4	+4	13	+9	13	0	5	–8	8	+3
24	Khanty-Mansi Autonomous Okrug–Yugra																						3	+3	4	+1	5	+1	3	–2	2	–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
26	Saratov Oblast														4	+4	7	+3	7	0	7	0	7	0	8	+1	10	+2	0	–10	6	+6
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
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
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
30	Orenburg Oblast																		3	+3	8	+5	9	+1	8	–1	11	+3	1	–10	4	+3
31	The Republic of Sakha (Yakutia)																				2	+2	4	+2	4	0	3	–1	0	–3	0	0
32	Primorsky Krai																														3	+3
33	Federal Biomedical Agency, Moscow														6	+6	11	+5	14	+3	16	+2	9	–7	5	–4	1	–4	1	0	3	+2
34	Federal Biomedical Agency, Krasnoyarsk																								24	Note	16	–8	8	–8	10	+2
	TOTAL in the Russian Federation	**225**	**300**	**+75**	**364**	**+64**	**381**	**+17**	**487**	**+106**	**470**	**–17**	**412**	**–58**	**420**	**+8**	**465**	**+45**	**434**	**–31**	**487**	**+53**	**564**	**+78**	**639**	**+74**	**732**	**+93**	**564**	**–168**	**652**	**+88**

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

Based on this indicator, the following regions lag behind other regions: Irkutsk Oblast (68.8%), Novosibirsk Oblast (70%), and the Republic of Tatarstan (72.9%).

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 2021, a total of 1,384 KT were performed (9.5 per million population). See Fig. 6.

Compared to the year 2020, the number of KT increased by 23.1% (+260).

New KT programs were launched in the Republic of Buryatia (Semashko Republican Clinical Hospital, Ulan-Ude), in Primorsky Krai (Primorsky Regional Clinical Hospital No. 1, Vladivostok), and in Ivanovo Oblast (Ivanovo Regional Clinical Hospital, Ivanovo).

There were 1,183 deceased-donor KT (8.1 per million population) in 2021, 201 (1.4 per million population) in 2020. See Fig. 6.

Table 6 and Fig. 7 show KT centers with the highest number of KT in 2021.

The rating primarily demonstrates the leadership and sustainability of transplant programs of 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 KT programs in Samara Oblast, in the Republic of Bashkortostan and Moscow Oblast, and further development of pediatric KT program in Shumakov National Research Center (Moscow) and 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 KT program, performing 114 transplants (56.7% of the total number of related KT in Russia).

In 2021, 6 KT centers performed more than 50 operations during the year: Shumakov Center (311), Sklifosovsky Research Institute of Emergency Care (231), Botkin City Clinical Hospital (87), Republican Clinical Hospital, Kazan (52), Belyaev Kemerovo Regional Clinical Hospital (51), and Research Institute of Urology (51). Five transplant centers performed from 30 to 49 operations during the year; 12 centers performed from 15 to 29.

In 2021, 21 transplant centers performed related-donor KT; a total of 201 transplants were performed. The average utilization of living kidney donation in 2021 was 14.5% of the total number of KT (13.4% in 2020).

Pediatric KT in 2021 were performed at 6 centers, and a total of 122 KT were performed (121 KT in 2020). Among the institutions performing it were Shumakov Center (61), Russian Children's Clinical Hospital (40), and National Medical Research Center for Children's Health (15); see Fig. 8.

EXTRARENAL ORGAN TRANSPLANTATION

In 2021, there were 290 HT (2.0 per million population) of which 15 were pediatric transplants and 2 heart-lung transplants (Shumakov Center).

Heart transplantations were performed in 17 centers. New HT programs were launched in 2 federal subjects of the Russian Federation:

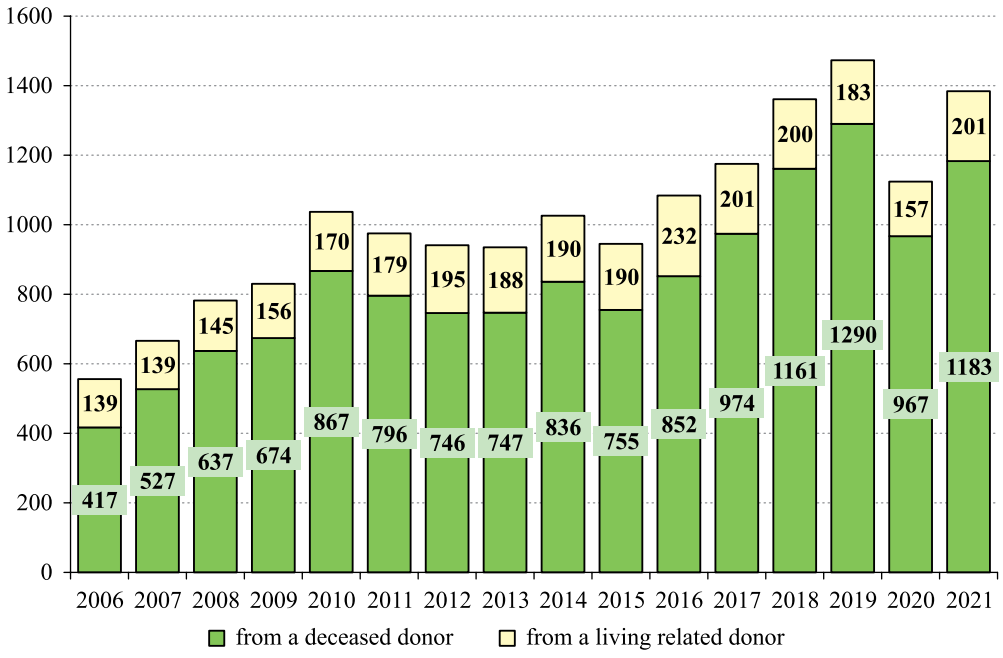

Fig. 6. Kidney transplantation in the Russian Federation in 2006–2021

– Tyumen Oblast (Regional Clinical Hospital No. 1, Tyumen),
– Khanty-Mansi Autonomous Okrug – Yugra (District Clinical Hospital, Khanty-Mansiysk).

Shumakov Center (Moscow) accounts for 72.9% (213, 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

Table 6

Leaders in terms of number of kidney transplants performed

Rank	Leaders in terms of number of kidney transplants performed	Number of kidney transplants in 2021
1	Shumakov National Medical Research Center of Transplantology and Artificial Organs, Moscow	283
2	Sklifosovsky Research Institute of Emergency Care, Moscow	231
3	Botkin City Clinical Hospital, Moscow	87
4	Republican Clinical Hospital, Kazan	52
5	Belyaev Kemerovo Regional Clinical Hospital, Kemerovo	51
6	Lopatkin Research Institute of Urology and Interventional Radiology – a branch of the National Medical Research Center for Radiology, Moscow	51
7	Samara State Medical University, Samara	47
8	Republican Clinical Hospital, Ufa	40
9	Vladimirsky Moscow Regional Research Clinical Institute, Moscow Oblast	40
10	Russian Children's Clinical Hospital, Moscow	40
	TOTAL	**922**
	66.6% of the total number of kidney transplants performed in the Russian Federation (1,384)	

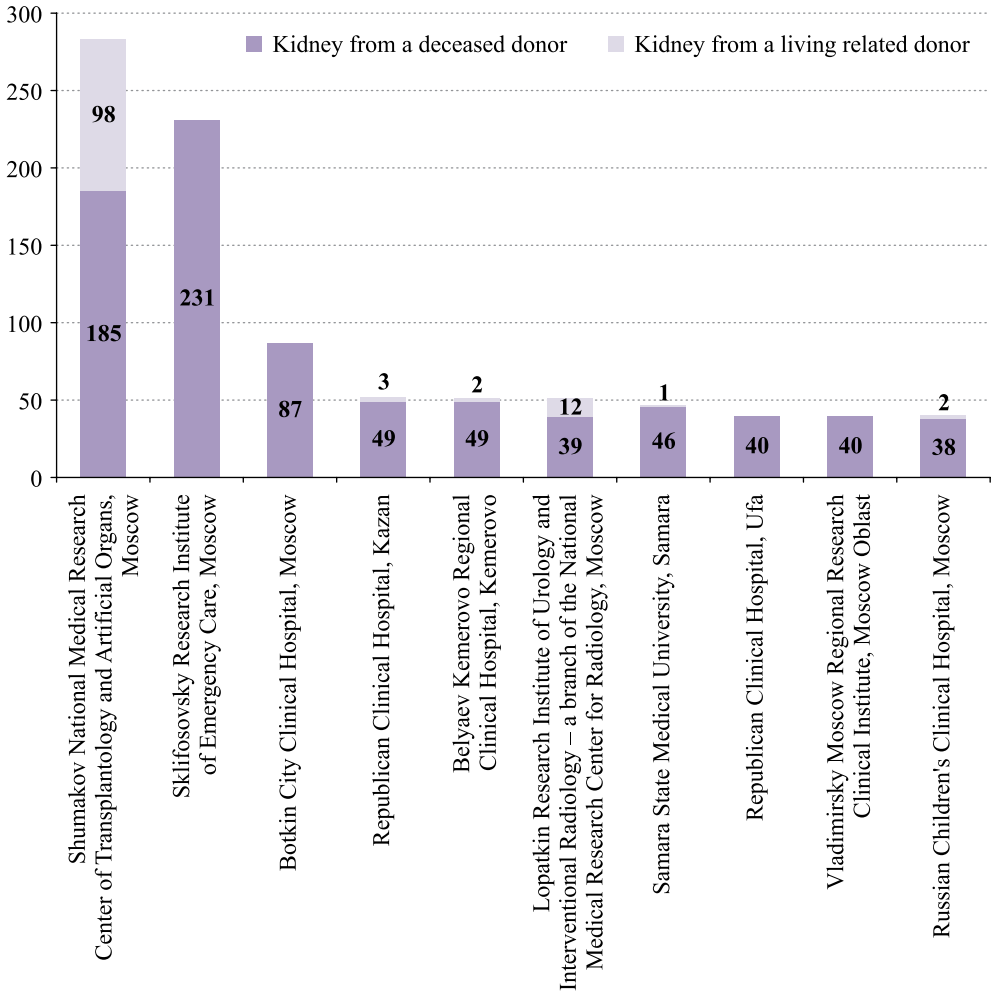

Fig. 7. Leaders in terms of number of kidney transplants performed

of this type of transplant care in the country. Apart from Shumakov Center, more than 10 HT in Russia are performed at Almazov National Medical Research Centre (22). Another 5 transplant centers performed from 5 to 8 HT: Sklifosovsky Research Institute of Emergency Care (Moscow), Ochapovsky Regional Clinical Hospital No. 1 (Krasnodar), Rostov Regional Clinical Hospital (Rostov-on-Don), Meshalkin National Medical Research Center (Novosibirsk), and Republican Clinical Hospital (Ufa). The remaining 10 (50.0%) performed less than 5 HT in the year.

LnT in 2021 were performed at 3 transplant centers. A total of 13 LnT and 2 heart-lung transplants were performed: 9 lung and 2 heart-lung transplants at Shumakov Center, 3 LnT at Sklifosovsky Research Institute of Emergency Care, and 1 LnT at Pavlov First St. Petersburg State Medical University.

Table 7 and Fig. 9 show the thoracic organ transplant centers that performed the highest number of heart-lung transplants in 2021.

In 2021, a total of 618 LiT (4.2 per million population) were performed, including 134 pediatric transplants. By comparison, there were 559 LiT (3.8 per million population) in 2020, of which 131 were pediatric transplants.

Liver transplants were performed at 29 centers. A new LiT program was launched in 2021 at Loginov Moscow

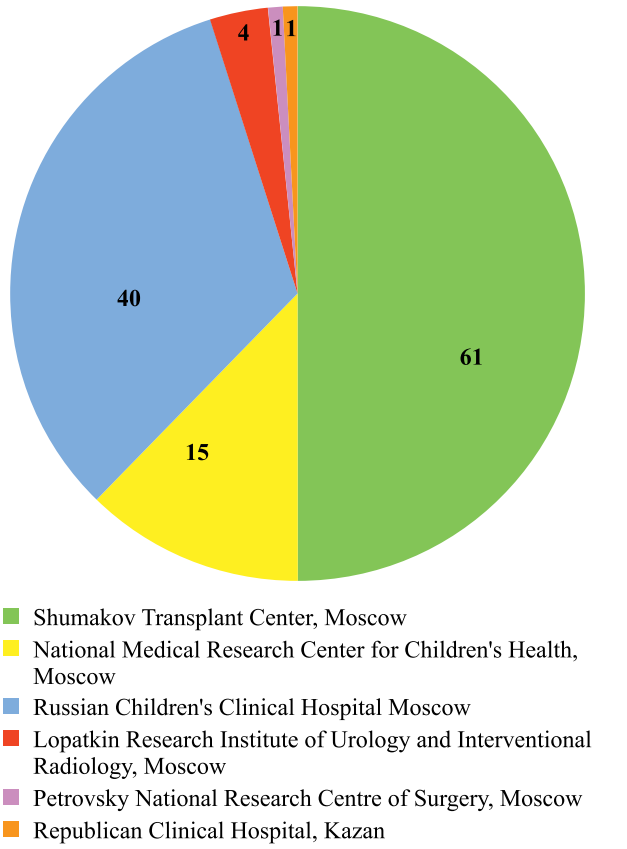

Fig. 8. Pediatric kidney transplantation in the Russian Federation in 2021

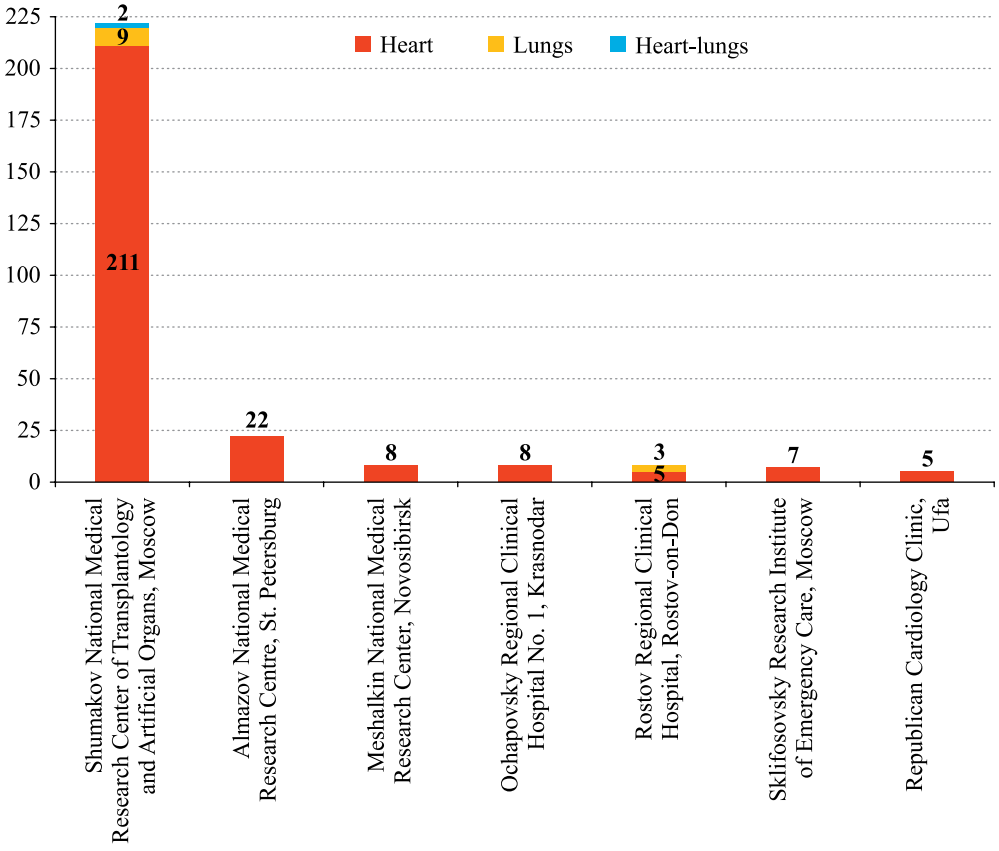

Fig. 9. Medical institutions that performed ≥5 heart transplants

Clinical Research and Practical Center, 6 deceased-donor LiT were carried out. In Republican Clinical Hospital, Kazan, the number of LiT increased to 28 (twice as many as in 2019).

In Russia, two transplant centers perform more than 100 LiT per year: the Shumakov Center (181) and the Sklifosovsky Research Institute of Emergency Care (122). Four other transplantation centers performed 20 or more LiT each: Burnazyan Federal Medical and Biophysical Center (41), State Novosibirsk Regional Clinical Hospital (47), Botkin City Clinical Hospital (40) and Republican Clinical Hospital, Kazan (28).

Transplant centers in Moscow and Moscow Oblast (8) accounted for 67.5% (417 transplants) of LiT in 2021; 69.8% (390 transplants) in 2020.

Table 8 and Fig. 10 show the LiT centers with the highest number of LiT in 2021.

The rating primarily demonstrates the leadership and sustainability of transplant programs of 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 living-related LiT. The positive dynamics of transplant programs in the Republic of Tatarstan and Novosibirsk Oblast, the leading role of pediatric living related LiT at Shumakov Center (Moscow), whose effectiveness (100 transplants) which exceeds the number of LiT in 8 of 10 transplant centers presented in this rating.

Related LiT were performed at 7 centers. Living-related transplants accounted for 163 surgeries (26.4%). In 2020, there were 9 centers that performed 169 related LiT (30.2%).

In 2021, 134 pediatric LiT were performed (mostly tender-age children); 131 in 2020. LiT were performed at 4 centers: Shumakov Center (119), Petrovsky National Research Centre of Surgery (8), State Novosibirsk Regional Clinical Hospital (6) and Kirov Military Medical Academy (1). This was the first time that pediatric LiT was being performed at Kirov Military Medical Academy.

Table 7

Medical institutions that performed ≥5 heart transplants

Rank	Centers that performed ≥5 heart transplants	Number of heart transplants in 2021
1	Shumakov National Medical Research Center of Transplantology and Artificial Organs, Moscow	213
2	Almazov National Medical Research Centre, St. Petersburg	22
3	Meshalkin National Medical Research Center, Novosibirsk	8
4	Ochapovsky Regional Clinical Hospital No. 1, Krasnodar	8
5	Rostov Regional Clinical Hospital, Rostov-on-Don	7
6	Sklifosovsky Research Institute of Emergency Care, Moscow	5
7	Republican Cardiology Clinic, Ufa	5
	TOTAL	**268**
	92.4% of the total number of heart transplants performed in the Russian Federation (290)	

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 2021
1	Shumakov National Medical Research Center of Transplantology and Artificial Organs, Moscow	181
2	Sklifosovsky Research Institute of Emergency Care, Moscow	122
3	State Novosibirsk Regional Clinical Hospital, Novosibirsk	47
4	Burnazyan Federal Medical and Biophysical Center, Moscow	41
5	Botkin City Clinical Hospital, Moscow	40
6	Republican Clinical Hospital, Kazan	28
7	Vladimirsky Moscow Regional Research Clinical Institute, Moscow Oblast	19
8	Rostov Regional Clinical Hospital, Rostov-on-Don	14
9	Volga Regional Medical Center, Nizhny Novgorod,	14
10	Granov Russian Research Center of Radiology and Surgical Technologies, St. Petersburg	12
	TOTAL	**518**
	83.8% of the total number of liver transplants performed in the Russian Federation (618)	

Pancreas transplants in 2021 were performed at 3 transplant centers: Sklifosovsky Research Institute of Emergency Care (7), Shumakov Center (2), and Petrovsky National Research Centre of Surgery (1). A total of 10 pancreas transplant surgeries were performed (16 in 2020), all of them being kidney-pancreas transplants.

One small bowel transplant was performed at Sklifosovsky Research Institute of Emergency Care.

Thus, there were 934 extrarenal transplants performed in 2021 or 40.3% of the total number of 2,318 (836, 42.6% in 2020). During the follow-up period from 2006 (106), the number of extrarenal organ transplants in the Russian Federation has increased by 828 (8.8-fold); see Fig. 11. Transplant centers in Moscow and Moscow Oblast accounted for 663 extrarenal organ transplants (71.0%) in 2021, which remains decisive.

Table 9 presents information on the number of organ transplants performed in the Russia Federation from 2006 to 2021.

ORGAN TRANSPLANT RECIPIENTS

According to information from the Federal Registry, there were 20,724 organ transplant recipients in Russia as of December 2021 (141.7 per million population); see Table 10.

During 8 years of observation, the number of organ recipients in the Russian Federation increased by 12,171 (142.3%). The number of KT recipients is estimated to be 13,059 (89.3 per million population); 3,902 (26.7 per million population) received liver, while 1,725 (11.8 per million) were HT recipients.

CONCLUSION

In 2021, donor and transplant programs were still under pressure from the COVID-19 pandemic. At the same time, the experience gained by medical organizations amidst the pandemic in 2020, mass vaccination of the population and the focus on providing the population with planned medical care in accordance with the state guarantee program, made it possible not only to maintain the volume of transplant care, but also to recover significantly from the drop in 2020 and reach the level of 2019.

In Moscow, donor activity exceeded that of pre-COVID 2019 (22.0), reaching 23.7 per million population. This allowed the Moscow Coordinating Center for Organ Donation to provide donor material to 10 Moscow-based transplant centers to perform 957 transplants.

The rate of increase in the number of effective donors and the number of organ transplants in 2021 was higher than envisaged by the departmental target program “Organ Donation and Transplantation in the Russian Federation”, approved via executive order No. 365 of the Russian Ministry of Health dated June 4, 2019.

Seven new organ donation and transplant programs were launched in 2021. They are:

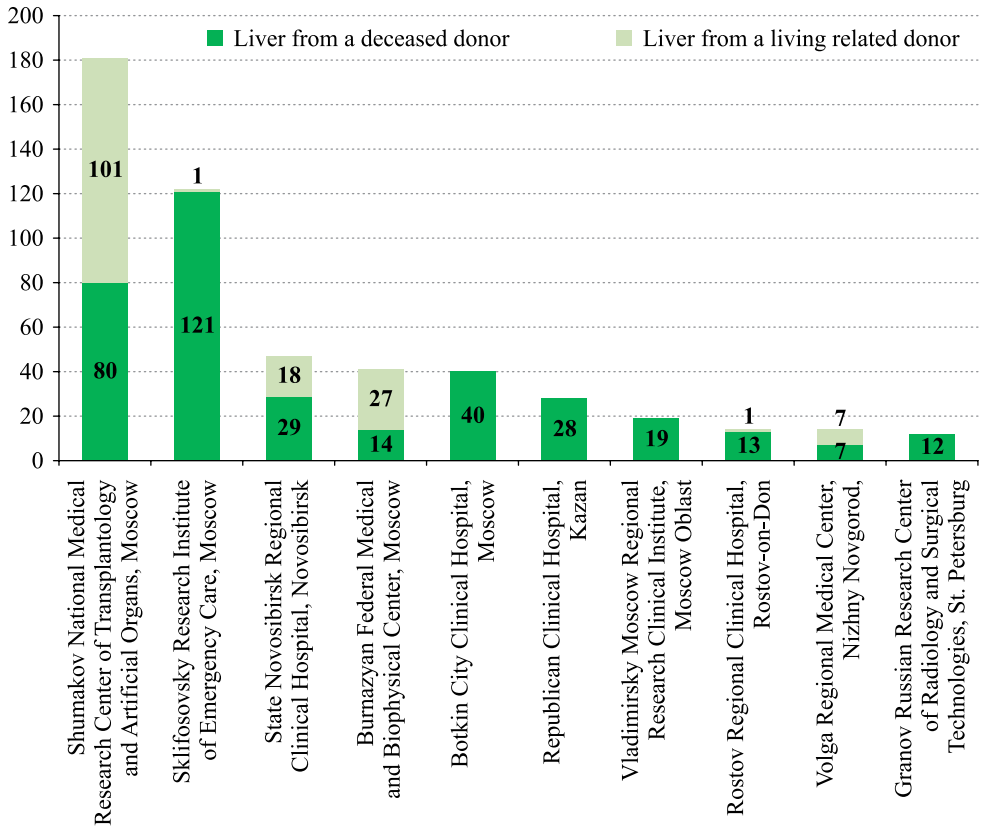

Fig. 10. Leaders in terms of number of liver transplants performed

– Semashko Republican Clinical Hospital, Ulan-Ude (living-related KT);
– Primorsky Regional Clinical Hospital No. 1, Vladivostok (deceased-donor KT);
– Ivanovo Regional Clinical Hospital, Ivanovo (deceased-donor KT);
– Loginov Moscow Clinical Research and Practical Center (LiT program);
– Regional Clinical Hospital No. 1, Tyumen (HT program);
– District Clinical Hospital, Khanty-Mansiysk (HT program);
– Kirov Military Medical Academy, St. Petersburg, (paediatric LiT program).

The number of pediatric transplants continued to increase in 2021, with 271 operations performed (227 in 2019 and 258 in 2020). The need for pediatric kidney and liver transplants is fully met; it is limited only by timely identification and referral of such patients to transplant centers for treatment; this also applies to adolescents in need of heart transplantation.

In 2021, it became possible to install an artificial left ventricle for children, including those with small anthropometric parameters, with end-stage heart failure, under the government guarantee program.

A record 618 liver transplants were performed in 2021 in the country; 163 of them were from living related donors.

The following are the prerequisites and plans for further development of donor and transplant programs in the federal subjects of the Russian Federation:

– Kemerovo Oblast, development of LiT program at Belyaev Kemerovo Regional Clinical Hospital;
– Ryazan Oblast and Tula Oblast, further development of organ donation and transplantation in the regions, interregional collaboration;
– Republic of Tatarstan, development of HT program, increasing the efficiency of donor kidneys utilization;
– Samara Oblast, implementation of liver and HT technologies in Samara State Medical University clinics, development of organ transplant program at Samara Regional Clinical Hospital;
– Novosibirsk Oblast, actualization of the regional organ donation program;
– St. Petersburg, further increase in transplantation care in accordance with the needs of the Northwestern Federal District, introduction of pediatric organ transplant program;
– Volgograd Oblast, implementation of HT technology at the Branch of Shumakov National Medical Research Center of Transplantology and Artificial Organs;
– Irkutsk Oblast, restoration of donor and transplant activity, introduction of heart transplant technology at Irkutsk Regional Clinical Hospital, increasing the efficiency of donor kidneys;
– Sverdlovsk Oblast, further increase in the volume of transplant care in accordance with the needs of the population;
– Saratov Oblast, relaunching of donor and transplant program, introduction of liver transplant technology;
– Krasnodar Oblast, restoration of donor and transplant activity in accordance with the real needs of the population and donor resource;
– Chelyabinsk Oblast, increasing donor and transplant activity;

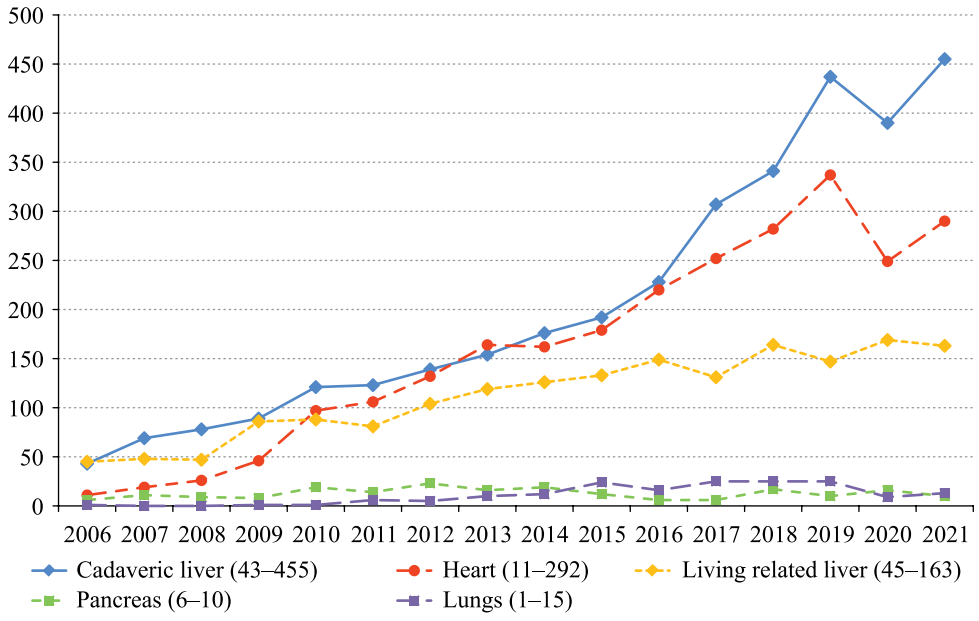

Fig. 11. Extrarenal organ transplant in 2006–2020

Table 9

Organ transplantation in the Russian Federation in 2006–2021

S/N.	Organ	2006	2007		2008		2009		2010		2011		2012		2013		2014		2015		2016		2017		2018		2019		2020		2021	
		Absolute number	Absolute number	Change over the year	Absolute number	Change over the year	Absolute number	Change over the year	Absolute number	Change over the year	Absolute number	Change over the year	Absolute number	Change over the year	Absolute number	Change over the year	Absolute number	Change over the year	Absolute number	Change over the year	Absolute number	Change over the year	Absolute number	Change over the year	Absolute number	Change over the year	Absolute number	Change over the year	Absolute number	Change over the year	Absolute number	Change over the year
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
2	*including cadaveric*	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
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
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
5	*including cadaveric*	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
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
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
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
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
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
11	Small bowel														1	+1	1	0	0	–1	0	0	0	0	0	0	0	0	1	+1	1	0
	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

Table 10

Number of organ transplant recipients in the Russian Federation in 2013–2021

ICD-10 code	Number of patients in the Registry (persons)																
	2013	2014		2015		2016		2017		2018		2019		2020		2021*	
		Absolute	Change (%)	Absolute	Change (%)	Absolute	Change (%)	Absolute	Change (%)	Absolute	Change (%)	Absolute	Change (%)	Absolute	Change (%)	Absolute	Change (%)
Z94.0 Kidney transplant status	6,651	7,502	12.8	8,164	8.8	9,063	11.0	9,658	6.6	10,851	12.4	11,880	9.5	12,563	5.7	13,059	–
Z94.1 Heart transplant status	416	520	25.0	639	22.9	803	25.7	952	18.6	1,164	22.3	1,355	16.4	1,524	12.5	1,725	–
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	1,150	1,406	22.3	1,649	17.3	1,948	18.1	2,152	10.5	2,632	22.3	3,032	15.2	3,489	15.1	3,902	–
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	1,135	24.9	1,344	18.4	1,497	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

* – The total number of patients with transplanted organs is provided from Register 14B3H, the number of patients with transplanted kidney, liver and heart is estimated, since it is calculated from the previous year based on the data on the number of organ transplants in 2021 and the data on the average survival of patients.

- Omsk Oblast, renewal of kidney donation and transplantation program in Omsk City Clinical Hospital No. 1.
- New organ donation and transplant programs are expected to be launched in Khabarovsk Krai, Kursk Oblast, and Yaroslavl Oblast.

The authors declare no conflict of interest.

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TRANSARTERIAL CHEMOEMBOLIZATION AND EARLY ARTERIAL COMPLICATIONS AFTER LIVER TRANSPLANTATION FOR HEPATOCELLULAR CARCINOMA

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Objective: to evaluate the possible influence of neoadjuvant transarterial chemoembolization (TACE) on development of early arterial complications after orthotopic liver transplantation (OLTx). **Materials and methods.** The work is based on treatment-related data of 250 recipients. The analyzed group included 21 patients with hepatocellular carcinoma (HCC). In all recipients who underwent primary transplantation (n = 228), possible negative factors influencing the development of early arterial complications were analyzed, such as degree of allograft steatosis, cold and warm ischemia time, revascularization duration, blood pressure level after arterial reconstruction, and exchange transfusion volume. **Results.** The degree of allograft steatosis did not differ between HCC patients and the general sample (95% CI, p = 0.25). No early arterial complications were revealed during TACE. There was no significant difference in preservation parameters, arterial revascularization time, systolic blood pressure level at blood flow start, and exchange transfusion volume (CI 95%, p > 0.05). The incidence of early vascular complications in the study group was 16.7%, it did not differ from the entire sample (95% CI, p = 0.96). **Conclusion.** The incidence of early arterial complications of OLTx in patients who underwent TACE does not significantly increase both according to the literature and our own findings. When vascular complications of OLTx occur, image-guided endovascular intervention is the method of choice for treatment.

Keywords: liver transplantation, neoadjuvant chemoembolization, early arterial complications.

INTRODUCTION

TACE is a recognized palliative treatment for HCC. In patients with HCC and cirrhotic transformation of the organ before a scheduled transplantation, neoadjuvant TACE reduces tumor size, biological activity of tumor and prolongs the waiting list time without significant progression. The literature describes specific arterial complications of embolization – intimal detachment, stenosis and thrombosis, leading to a shortage in blood supply to the organ, development of early complications, including in patients with subsequent liver transplantation [2, 3, 5].

MATERIALS AND METHODS

The work is based on the data of 250 recipients. The analyzed group included 21 patients with HCC, 9 men and 12 women aged 27.9 to 64.6 years (mean age was 49.7 ± 7.48). In absolute majority, HCC resulted from chronic viral hepatitis and cirrhosis. To assess the impact of neoadjuvant TACE on early arterial complications in all primary transplant recipients (total group, n = 228), possible adverse events were analyzed. In recipients on the waiting list who underwent TACE, these were intimal dissection and aneurysm, impaired arterial perfusion of the organ, and arterial thrombosis during arterial hepatography. Eighteen recipients underwent 1 to 7 courses of neoadjuvant TACE, including four cases that combined pre-transplant liver resection. In two cases, radiofrequency ablation and liver resection without TACE were done.

Only one patient did not receive neoadjuvant therapy.

Before neoadjuvant TACE, 7 of 21 potential candidates for OLTx did not meet the Milan criteria.

After arterial hepatography and reverse portography, embolizumab, a suspension of oil contrast (lipiodol 4 to 10 mL) and antitumor drugs (doxorubicin 40–60 mg, mitomycin 10–15 mg) or 70 mg doxorubicin in saturable spheres, was injected superselectively into vessels feeding the tumor (DEB, patients 4, 8, and 9). The procedure may have been supplemented by mechanical occlusion of the hepatic artery branches feeding the tumor with a fine-cut hemostatic sponge. There were no immediate complications of the procedure. Length of stay in the hospital for TACE ranged from 3 to 9 days (5 days on average). Subsequent liver transplantation after neoadjuvant treatment was performed within 2 days to 10 months.

In all cases, cold perfusion during allograft preparation was performed with custodiol solution (HTK "Custodiol", Kohler, Germany) in 8–14 liters. Subsequently, during liver transplantation, we recorded the preservation parameters – cold and warm ischemia

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time; arterial revascularization duration; blood pressure after arterial reconstruction. A physical intraoperative assessment was carried out – the presence of pulsatile flow distal to the formed anastomosis, volumetric flow rate was determined by Doppler ultrasound flowmetry. An at least 100 mL/min volumetric flow rate was considered adequate. Exchange transfusion volume was determined by mathematical addition of transfused donor and collected autoerythrocyte masses using a cell saver machine. The period between the last TACE and organ transplant surgery was also evaluated. Outcomes of repeated transplants (n = 22) were excluded from the study.

RESULTS

In the sex and age distribution, HCC patients who underwent neoadjuvant TACE did not differ significantly from other recipients ($p > 0.05$ in both samples). The level of macrovesicular steatosis of the allograft prepared for transplantation did not differ between the HCC patient groups and the overall sample (95% CI, $p = 0.25$). Among the seven patients who did not initially meet the Milan criteria, neoadjuvant TACE was performed in six cases, which in half of the cases reduced the tumor size to the above criteria. There was a significant decrease in the median levels of alpha-fetoprotein before and after comprehensive neoadjuvant treatment – from 86 to 23.6 IU/mL ($p < 0.05$).

There were no deaths from early arterial complications of OLTx in the study group. There were also no angiographic complications – intimal injury, reduced hepatic arterial perfusion during a postponed study, and hepatic artery thrombosis in waitlisted patients.

Average cold ischemia time in the analyzed group was 377.2 minutes, whereas the overall time for all transplants was 397.8 minutes; warm ischemia time was 54 and 47.8 minutes, respectively ($P > 0.05$). At arterial blood flow start, there was systolic blood pressure below 100 mm Hg in 41.7% of 228 recipients, and in 33.3% of the analyzed group. No significant difference affecting the development of early complications (95% CI, $p = 0.49$) were found. Exchange transfusion volume was comparable in all patients and those who underwent neoadjuvant treatment (Table 2).

Three patients in the study group (16.7%) had early vascular complications. In one case, blood supply deficiency was corrected intraoperatively by forming an anastomosis with the aorta (patient #16). In a combination of hepatic artery stenosis of the graft and steal syndrome (patient #14), a successful balloon angioplasty of arterial anastomosis and splenic embolization were performed four days later; in one patient, the steal syndrome was eliminated by splenic embolization on day 6 after OLTx (patient #18).

We should note a case of late hepatic arterial thrombosis with the development of necrotizing cholangitis, which was observed at day 32 and was not related to the TACE procedure and OLTx technique (patient #6).

Table 1

Neoadjuvant therapy options for patients on the waiting list

S/N	Patient, age in years	Diagnosis, stage	Secondary diagnosis	Previous treatment
1	K., 27	HCC T4N0M0		4 TACE
2	U., 48	HCC T3N0M0	PBC	1 TACE
3	K., 49	HCC T3N0M0	CHBI	2 TACE
4	K., 43	HCC T2N0M0	CHBI	1 TACE (DEB)
5	E., 49	HCC T3N0M0	CHCI	3 TACE
6	K., 53	HCC T2N0M0	CHBI	4 TACE, open radiofrequency ablation
7	S., 60	HCC T2N0M0	CHCI	2 TACE
8	R., 44	HCC T1N0M0	CHBI	1 TACE (DEB)
9	Z., 54	HCC T3N0M0	CHCI	2 TACE (DEB)
10	K., 52	HCC T2N0M0	CHCI	7 TACE
11	B., 58	HCC T3N0M0	CHCI	2 TACE, video laparoscopic resection, Radiofrequency ablation
12	N., 54	HCC T2N0M0	CHCI	5 TACE
13	T., 44	HCC T2N0M0	CHCI	2 TACE
14	S., 47	HCC T2N0M0	CHCI	2 TACE/TIPS
15	P., 52	HCC T3N0M0	CHCI	6 TACE, video laparoscopic resection
16	S., 64	HCC T3N0M0	PBC	2 TACE, video laparoscopic resection
17	P., 42	HCC T2N0M0	CHCI	None
18	P., 53	HCC T2N0M0	CHCI	2 TACE
19	R., 46	HCC T1N0M0	CHCI	Radiofrequency ablation
20	S., 46	HCC T3N0M0	CHBI	Video laparoscopic resection
21	K., 44	HCC T2N0M0	CHCI	1 TACE

Thus, in the early post-OLTx period, incidence of vascular complications was 16.2% in all patients and 16.7% in recipients who received neoadjuvant TACE. There was no significant difference (95% CI, p = 0.96).

One-year graft survival (Kaplan–Maier estimates) was 91% (Fig.).

DISCUSSION

Arterial and subsequent biliary complications are the main cause of graft dysfunction and patient death after OLTx. The severity of early vascular complications after liver transplantation is incomparable with the general surgical problems of the early postoperative period. Hepatic artery thrombosis leads to severe graft dysfunction and loss, which explains the high mortality rates. Arterial stenosis and arterial kink, and steal syndrome are the cause of arterial insufficiency in a transplanted organ, they determine the subsequent formation of multiple biliary strictures, deterioration of its function and recipient's condition, which eventually requires retransplantation in 20–40% of cases [9, 3].

Today, TACE is regarded as the standard of care for patients with HCC against a background of cirrhosis. It allows to achieve "a decrease in the stage of the disease". It supports the status of their stay on the waiting list, including the Milan criteria [4]. Our experience shows superselective TACE allows 60% of potential candidates to prolong their stay on the waiting list for one year without significant progression [6, 8].

Severe complications of TACE in the form of intimal dissection and acute hepatic artery thrombosis usually occur when performing lobular embolization and with little experience in such procedures. Our research center performs several hundred chemoembolizations in patients with malignant liver tumors annually. Mortality is less than 2%, and the side effects do not require surgical intervention [7].

Current literature discusses the possible relationship of early vascular complications of OLTx in patients after neoadjuvant TACE. For example, D. Sneiders et al. analyzed the outcomes of OLTx after TACE in 1,122 patients in 14 retrospective studies. Both vascular and biliary complications of OLTx were studied. All patients only after TACE with doxorubicin were included, but the technique itself was not considered: the authors in this meta-analysis did not separate classical oil TACE and DEB; they allowed lobular embolization in multiple sources of blood supply. There was increased incidence

Table 2

Comparative indicators and differences by groups

Parameters (mean values)	General group (n = 228)	Analyzed group (n = 18)	P
Cold ischemia, minutes	397.8	377.2	>0.05
Warm ischemia, minutes	47.8	54	>0.05
Arterial revascularization, minutes	48.3	56	>0.05
BP level at the time of arterial start, <100 mm Hg/%	41.7	33.3	<0.5
Exchange transfusion volume, mL	1520	1572.8	>0.05

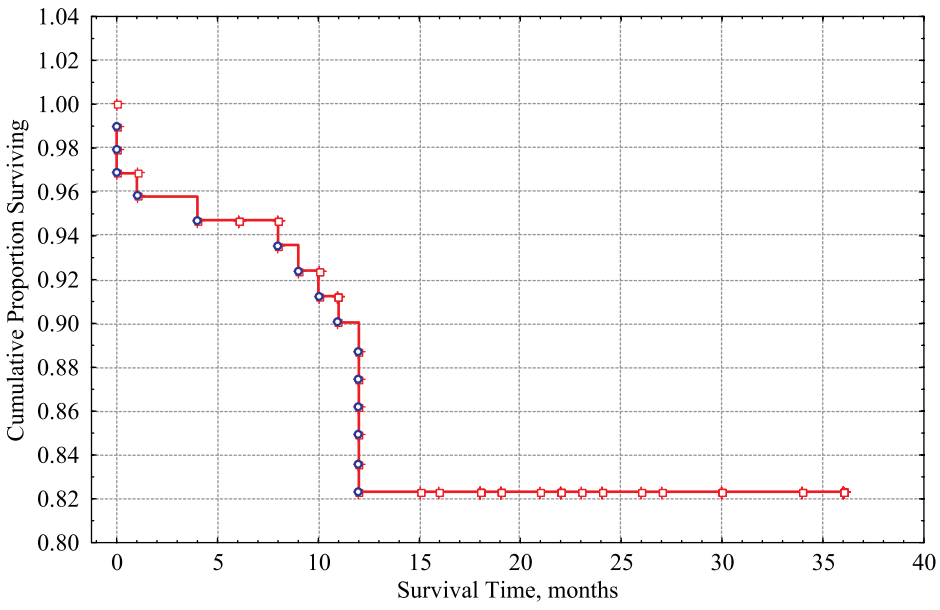

Fig. Cumulative graft survival (Kaplan–Maier)

of vascular complications of OLTx after TACE, but none of them were significant (p = 0.02) [3]. At the same time, achievement of TACE effect in patients with advanced HCC against the background of cirrhosis is a "bridge" to OLTx. Their survival is comparable to that of recipients who meet the Milan criteria; in addition, TACE potentially reduces the risk of relapse and progression [3, 5, 8].

A number of publications have studied histological changes in the wall of the lobar hepatic arteries of the explant. Panaro F. et al. detected arterial wall edema, fibrosis and hemorrhagic intimal necrosis in 12 of 32 patients with neoadjuvant TACE. However, there was no significant difference in the number of vascular complications of OLTx (28% each in the TACE group and control group) and mortality from graft loss (6.25% vs 5.75%) (p = 0.01) [4].

At our research center, TACE in HCC patients on the OLTx waiting list is performed using microcatheter technique only, always superselectively by definition. After performing 49 TACE procedures in 18 patients from the OLTx waiting list at our center, no consequences were noted [6, 8]. The incidence of vascular complications of transplantation was not significant – 16.2% in all patients and 16.7% in recipients who received neoadjuvant TACE (p = 0.96).

CONCLUSION

The incidence of early arterial complications of OLTx in TACE recipients, according to literature and in our observations, has not increased.

Achieving the effect of the ongoing neoadjuvant treatment in patients with advanced stages of HCC against a background of cirrhosis is a "bridge" to OLTx. Patient survival is comparable to that of recipients who met the Milan criteria.

In the case of vascular complications of OLTx, the treatment method of choice is X-ray image-guided endovascular interventions.

The authors declare no conflict of interest.

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DE NOVO HEPATITIS B VIRUS INFECTION AFTER LIVER TRANSPLANTATION

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De novo hepatitis B virus (HBV) infection developing after liver transplantation (LTx) is the development of infection in a patient with liver disease etiologically unrelated to HBV infection and who had no preoperative HBV markers. **Objective:** to analyze the clinical features and characteristics of *de novo* HBV infection and evaluate the efficacy of nucleos(t)ide analogue therapy in liver transplant recipients. **Materials and methods.** The study involved 247 adult patients who underwent deceased donor LTx from 2016 to 2022 at Shumakov National Medical Research Center of Transplantology and Artificial Organs and who had no pre-transplant HBV markers. **Results.** Twenty-two (7%) of 247 patients had *de novo* HBV markers from 5 to 69 months. At the time HBV DNA was detected, the mean alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels in the patients was 53.3 ± 36.4 IU/L and 54.5 ± 33.0 IU/L, respectively. All patients received nucleos(t)ide analogues (NAs). The therapy led to a statistically significant decrease in the mean ALT level to 31.5 ± 24.2 IU/L ($p = 0.049$) and AST to 33.33 ± 21.5 IU/L ($p = 0.025$). In most cases (18 persons, 81%), no serum HBV DNA was detected after treatment (6 ± 3 months). **Conclusion.** Timely detection of *de novo* HBV risk factors, early diagnosis and immediate treatment can prevent severe graft damage.

Keywords: HBV infection de novo, liver transplantation, nucleos(t)ide analogues, entecavir, tenofovir, immunoglobulin.

INTRODUCTION

De novo HBV infection arising after LTx is the development of infection in a patient/recipient with liver disease that is etiologically unrelated to HBV infection and who had no preoperative HBV markers. According to reports, *de novo* HBV infection after orthotopic LTx in patients without viral replication and even in patients without markers of previous infection with HBV is between 1.7% and 5% [1]. Untreated HBV infection leads to severe liver disease, rapid graft dysfunction, graft cirrhosis, and risk of hepatitis D virus (HDV) co-/superinfection.

OBJECTIVE

To analyze the clinical features and characteristics of *de novo* HBV infection and evaluate the efficacy of therapy with nucleos(t)ide analogue therapy in liver recipients.

CLINICAL CASES AND RESEARCH METHODS

The study involved 247 adult patients who underwent deceased donor LTx from 2016 to 2022 at Shumakov National Medical Research Center of Transplantology and Artificial Organs and who had no pre-transplant HBV markers. After LTx, the patients underwent standard clinical examination at least once every 3 months, including interview and examination, routine laboratory – total blood count, biochemical blood count, coagulogram, total urine count, measurement of immunosuppressive drug levels in blood, serological blood test – hepatitis C antibodies, hepatitis B surface antigen (HBsAg) – and instrumental examinations (abdominal ultrasound. When a positive HBsAg was detected, we performed qualitative and quantitative detection of HBV DNA by polymerase chain reaction (PCR), and examined the HBV profile (HBeAg, anti-HBe, HbcAg, anti-HBc IgM) and HDV antibodies. All patients received immunosuppressive therapy in various combinations. When HBV infection markers were detected, patients were prescribed high-barrier NAs – entecavir (ETV) and tenofovir (TDF).

Statistical analysis was performed using Statistica 12.6 software. Differences were considered statistically significant at the $p < 0.05$ level.

RESULTS

Of 247 patients, 22 (7%) (8 men and 14 women) showed *de novo* HBV infection markers (HBV DNA, HBsAg) at 5 to 69 months (mean was 21.4 ± 17.3 months, median was 17 months). No cases of HDV co/superinfection were identified.

The most common indications for LTx were cirrhosis resulting from autoimmune liver diseases (autoimmu-

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ne hepatitis (AIH), primary biliary cholangitis (PBC), primary sclerosing cholangitis (PSC)), hepatitis C virus (HCV) and toxic liver disease. Other indications for transplantation were renal allograft dysfunction (retransplantation due to recurrent underlying disease – 1 AIH and 1 PSC), hepatic alveolar echinococcosis (HAE), Byler disease and neuroendocrine liver metastases (NELM). One patient underwent simultaneous liver and kidney transplantation due to polycystic liver/kidney disease (Table 1).

In one patient, HBV infection developed 248 months after LTx, which resulted in graft injury requiring retransplantation. In the remaining patients, HBV infection proceeded without severe clinical manifestations.

Patients continued to receive immunosuppressive therapy to the same extent as before the detection of *de novo* HBV infection markers. Most patients received double immunosuppressive therapy (10 patients, 45%) or tacrolimus (TAC) monotherapy (8 patients, 36%); 18% had a triple immunosuppressive protocol (Table 2). Mean whole blood TAC concentration was 6.05 ± 2.01 ng/mL.

At the time HBV DNA was detected, patients had 53.3 ± 36.4 IU/L and 54.5 ± 33.0 IU/L as mean ALT and AST, respectively. All patients with positive HBV DNA were prescribed the high-barrier NAs – ETV and TDF; three patients were initially treated with ETV, then converted to TDF disoproxil fumarate due to persistent viremia. One patient received lamivudine (LVD), which was subsequently changed to ETV because of resistance to LVD (Table 3).

The therapy led to a statistically significant decrease in the mean ALT level to 31.5 ± 24.2 IU/L ($p = 0.049$) and AST to 33.33 ± 21.5 IU/L ($p = 0.025$). In most cases (18 persons, 81%), no serum HBV DNA was detected after 6 ± 3 months of treatment. Also, 10 (45%) patients had HBsAg seroconversion after 19.7 ± 9.5 months. Of these, 7 received ETV therapy and 3 received TDF. Twelve patients (54%) remained HBsAg-positive in the absence of viremia. Two patients (9%) were treated with NAs for no more than 4 months and had a viral load of 8.0×10^3 IU/mL.

Table 1

Underlying diseases leading to liver transplantation in *de novo* HBV infection

Underlying disease	Patient count
AIH	4
HCV	3
PBC	3
PSC	3
Toxic hepatitis	2
RAD	2
HAE	1
Polycystic liver disease	1
Wilson–Konovalov disease	1
NELM	1
Byler disease	1

Table 2

Immunosuppressive therapy in patients with *de novo* HBV infection

Therapy regimen	Patient count
TAC	8
TAC + MMF	7
TAC + MMF + Methylprednisolone	4
TAC + Everolimus	2
TAC + Methylprednisolone	1

Note: MMF, mycophenolic acid / mycophenolate mofetil.

Table 3

Antiviral therapy in patients with *de novo* HBV infection

Drug	Patient count
ETV	14
TDF	3
ETV → TDF	3
Tenofovir alafenamide (TAF)	1
LVD → ETV	1

DISCUSSION

As in the general population, the source of HBV infection may be blood transfusions, surgical interventions, including dental surgeries, etc. Accordingly, HBV infection markers can be detected during the whole life of a recipient, which our study demonstrates – the average time before the onset of infection was almost two years. In our sample, the prevalence of *de novo* HBV infection was consistent with literature data [2].

HBcAb- and HBsAb-negative recipients are at the highest risk of *de novo* HBV infection [3]. A recent study reported that of 1,458 patients, 21 (1.4%) were found to have *de novo* HBV infection. The time to detection of infection varied, ranging from 8 to 55 months. HBcAb-negative recipients had a higher risk of *de novo* HBV infection than HBcAb-positive recipients (22.6% versus 9.1%). The incidence of *de novo* HBV infection did not differ depending on the recipient's HBs-antibody status [4].

There are three main approaches to prevent *de novo* HBV infection: active immunization (vaccination of recipients before liver transplantation), passive immunization (administration of human hepatitis B immune globulin, HBIg) and therapy with direct antiviral drugs (nucleos(t)ide analogues) for preventive purposes at high risk of infection and for treatment when infection markers are identified.

There are ongoing studies looking at active immunization of liver transplant recipients before and after LTx with monitoring of HBs antibody titers as a measure

to prevent *de novo* HBV infection. One was presented in 2017; in this study, Wang et al. looked at a group of 71 liver recipients who received HBV vaccination before and after transplantation from HBcAb-positive donors. The mean follow-up period was 8 years, with only 3 (4%) cases of *de novo* HBV infection reported. All patients belonged to the group with insufficient immune response to vaccination (anti-HBs titer of <100 IU/L). The detected infection had no significant abnormalities in the biochemical blood count that would have required liver biopsy, and it had no effect on the transplant outcome. Throughout the study, a fairly large number of vaccine injections were required to maintain immunity (average of 4 doses; range 1–9 doses), and 9 patients were never vaccinated after transplantation because of contraindications. Thus, the researchers note that the approach described is cheaper, but the vaccines are less effective in cirrhosis and require careful monitoring of response after vaccination. Vaccination timing is difficult to predict, and vaccination can take months, and some patients fail to achieve target anti-HBs levels for a variety of reasons. This approach is more applicable in the context of living donor liver transplantation, when surgery is performed routinely [5].

As in patients with initial HBV infection, human anti-HBsAg immune globulin (HBIg) is used in patients with *de novo* HBV infection. Some studies indicate the effectiveness of HBIg monotherapy with a very low risk of *de novo* HBV infection in recipients who have received a transplant from an anti-HBc-positive donor and who have an anti-HBs titers of >100 IU/L [5]. However, the lack of long-term data, the risk of decreasing anti-HBs titers over time, and the need for concomitant antiviral prophylaxis in nonresponders have led to a significant reduction in the use of this strategy. According to several studies, extra addition of HBIg to NAs administration did not enhance treatment efficacy [6, 7]. The American Association for the Study of Liver Diseases and the European Association for the Study of the Liver recommended nucleos(t)ide analogue monotherapy for prevention and treatment of *de novo* HBV infection, given the absence of differences in treatment outcomes with and without HBIg, low frequency of *de novo* HBV infection, high cost of immunoglobulin, and the need for intravenous routes for administration of this medication [8].

For many years, LVD was the standard treatment for HBV infection, with approximately 3% of patients developing the infection despite taking the drug [9]. Subsequently, various primary and secondary mutations leading to resistance to LVD treatment have been identified. The most common primary mutations associated with LVD resistance occur in codon 204 in the tyrosine-methionine-aspartate-aspartate (YMDD) site and result in amino acid substitution – rtM204V/I (replacement of methionine with valine or isoleucine). These changes cause >100-fold decrease in sensitivity to LVD [10]. Resistance to LVD develops gradually during treatment: with a rate of 14% to 32% in the first year of treatment and exceeding 70% after 48 months of therapy [11]. There was also data in the literature on the effectiveness of tenofovir [12]. However, currently, due to the high rate of resistance, the need for long-term prevention/treatment and the development of a number of side effects, high-barrier NAs – ETV, TDF and TAF – are used in clinical practice [6, 7, 13].

In our study, we used the most modern treatment regimen for HBV-infected patients – high-barrier reverse transcriptase inhibitors, NAs – ETV and TDF salts. Thanks to timely administration of these preparations, *de novo* HBV infection was, in the overwhelming majority of cases, mild, without clinical manifestations and with minimal changes in laboratory values (ALT and AST increased to 2–2.5 norms at most). The therapy led to a statistically significant decrease in hepatic aminotransferases, and all patients had an undetectable level of HBV DNA by PCR. HBsAg seroconversion was observed in 45% of cases. Our results correlate with other studies. One of the most voluminous works was published in 2021 by Saidy et al. out of 2686 liver transplant recipients, 32 patients (1.2%) demonstrated a *de novo* HBV infection without an obvious source of infection. Additionally, 78 (2.9%) received a HBcAb-positive graft without having undergone HBV-infection prior to LTx. In this subgroup, 14 (17.9%) patients were recorded with *de novo* HBV infection. After the diagnosis, the patients were treated with either ETV or tenofovir. The authors noted a significant reduction in inflammation signs and no progression of steatosis on graft biopsy after initiation of therapy; no difference in survival between patients with and without *de novo* HBV infection was found [14]. Consequently, timely detection of risk factors for *de novo* HBV infection, early diagnosis and immediate initiation of treatment can prevent serious damage to the graft, which has been confirmed in various studies.

CONCLUSIONS

1. The clinical course of *de novo* HBV infection in the examined patients was mild with minimal clinical and laboratory manifestations. Therefore, timely detection and initiation of antiviral therapy will increase graft survival in this patient cohort.
2. Antiviral therapy with NAs is effective against the background of immunosuppressive therapy and is accompanied by disappearance of replication markers in the majority of patients (81%) 6 ± 3 months after the beginning of antiviral therapy; HBsAg seroconversion was observed in 45% after 19.7 ± 9.5 months.
3. High-barrier NAs are effective enough; additional therapies are not required.

The authors declare no conflict of interest.

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USE OF ENDOSCOPIC BAND LIGATION ALONE AND IN COMBINATION WITH NONSELECTIVE BETA BLOCKERS FOR PREVENTION OF VARICEAL BLEEDING IN ASCITES PATIENTS ON THE LIVER TRANSPLANT WAITING LIST

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Objective: to conduct a comparative analysis of the effectiveness of two methods – endoscopic band ligation (EBL) alone and in combination with nonselective beta blockers (NSBB) – used for prevention of variceal bleeding (VB); to evaluate their impact on patient survival in severe ascites during long-term stay on the liver transplant waiting list (LTWL). **Materials and methods.** A retrospective comparative study of two groups of patients with decompensated liver disease, ascites and varices included in the LTWL, who received EBL (n = 41, group 1) and EBL + NSBB (n = 45, group 2). **Results.** The groups being compared did not differ in demographics, clinical parameters, MELD and Child–Turcotte–Pugh scores. There were no significant differences in the incidence of severe ascites, particularly diuretic-resistant ascites. The study groups did not differ in the incidence of medium- and large-sized varices. Incidence of bleeding did not differ in both groups. Overall mortality was significantly higher in the EBL + NSBB group than in the EBL group. Patient survival was lower, while mortality was higher in the EBL + NSBB group. The combined therapy group had a significantly higher number of acute kidney injury (AKI) than the EBL group. **Conclusion.** The compared methods are equivalently effective in preventing VB in patients with decompensated cirrhosis with a prolonged stay on the waiting list. Survival rate is significantly lower, while mortality is significantly higher in the EBL + NSBB group than in the EBL group.

Keywords: liver transplant waiting list, ascites, bleeding, nonselective beta blockers, endoscopic band ligation.

INTRODUCTION

The introduction of various types of liver transplantation (LTx) into clinical practice has made irreversible liver diseases highly curable. LTx has become the therapy of choice for end-stage liver diseases, acute liver failure and selected cases of hepatocellular carcinoma (HCC) [1]. Decompensated cirrhosis is one of the main indications for LTx [2, 3]. Increase in the number of liver transplantations due to expanded indications, as well as a significant increase in potential recipients on the liver transplant waiting list (LTWL), which have been witnessed worldwide in recent years, have led to an acute problem of organ (liver) shortage in almost all countries of the world [4]. Acute shortage of liver donors has raised the challenge of preserving life and preventing dropout of patients from the LTWL. Portal hypertension (PH) is a major complication of cirrhosis, characterized by increased pressure in the portal venous system, leading to portosystemic collateral vasculature [5, 6]. Dilated veins of the esophagus and stomach constitute a real clinical problem due to their possible rupture with subsequent catastrophic bleeding [5], which is the main cause of death in patients with cirrhosis, including those waiting for LTx [7]. The prevalence of esophageal varices (EV) varies between 40% and 95% in patients with cirrhosis [8, 9]. The annual detection rate of EV in patients with clinically significant portal hypertension (CSPH) varies from 3% to 22% [10–12]. Approximately 15–20% of patients with cirrhosis develop bleeding within 1 to 3 years [13, 14]. In short-term follow-up, the mortality rate in the event of a VB episode varies from 15% to 30% [15–18].

The 5-year VB-associated mortality in patients with cirrhosis is over 80% [19]. Mortality due to VB is usually determined by size of varices or basal liver function [20]. According to the Baveno VI guideline, 2 major axes of primary prophylaxis for varices are suggested: NSBB and EBL [21]. EBL is a physical method that rarely causes hemodynamic changes. On the contrary, NSBB can induce hemodynamic changes by reducing cardiac output (CO) and vasodilation [22]. In this context, it is unclear whether the use of NSBB is actually beneficial for end-stage liver disease [21, 23].

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Serste et al. [22] first showed the risk of NSBB use in this category of patients and proposed the "therapeutic window" hypothesis for NSBB use, considering the optimal use of this class of drugs in cirrhosis progression [20]. These researchers concluded that NSBB should be used with caution in decompensated cirrhosis. Among the unresolved problems that would confirm their usefulness is the use of NSBB in decompensated liver or in refractory ascites (RA).

Ascites is one of the most common complications of cirrhosis. In the practice of physicians managing patients in the LTWL, cases of simultaneous development of ascites and VB is not uncommon. In patients with different etiologies of cirrhosis, CSPH is the main driver of complications such as ascites or VB [23]. It is unknown whether NSBB is useful or, on the contrary, dangerous for patients with ascites and VB.

An important aspect of this problem is that in most works containing optimistic results, the effectiveness of NSBB was evaluated in the short term, on average about 6 months [24, 25]. Taking into account the fact that the average patient survival in VB is about 2 years, it is very difficult to interpret the above results with a positive outcome of NSBB in the short-term management period on a population of LTWL patients for 2 years or more.

In this regard, the **objective** of this work was to compare the effectiveness of two methods (EBL alone and EBL plus NSBB) used for prevention of VB so that their impact on the survival of patients with severe ascites during long-term stay in the LTWL could be evaluated.

MATERIALS AND METHODS

The study was conducted at the Center for Surgery and Donor Coordination, Rostov Regional Clinical Hospital. It was approved by the local ethics committee. The analysis included data from 86 waitlisted patients with cirrhosis of various etiologies (viral, alcoholic). EV was detected via screening endoscopy, which led to us preventing bleeding in 45 patients by prescribing NSBB (carvedilol, propranolol, nadolol) in combination with EBL; in 41 patients, EBL was used without subsequent prescription of NSBB. Patient demographic and clinical data were obtained from a continuously updated electronic database.

Inclusion criteria: presence of EV, grade 2 or 3 ascites by the time of initiation of VB prophylaxis.

Exclusion criteria: patients with HCC or other malignancies with the development of ascites, patients who have used NSBB for <4 weeks, patients who have undergone LTx, patients with heart rate <60/min and/or systolic blood pressure (SBP) <90 mmHg.

MELD-Na [26] and Child–Turcotte–Pugh [27, 28] scores were calculated. Ascites severity was determined in accordance with the International Ascites Club guidelines [29]. During screening endoscopy, EV with high risk of bleeding were determined and named "varices needing treatment" ("VNT") according to Baveno VI [21] and World Gastroenterology Organisation (WGO) criteria [30] in the foreign literature. Advanced diagnostic criteria of the International Ascites Club were used to diagnose AKI in cirrhosis [31].

In waitlisted patients with alcoholic cirrhosis, abstinence confirmed by narcologists and psychiatrists was maintained for at least 3 months. Patients with cirrhosis associated with HBV and HCV infections received antiviral therapy with nucleoside analogues and a combination of direct-acting antivirals, respectively. All patients in the LTWL underwent clinical and biochemical investigations; their hemostasis parameters were examined. When the patients were stable, blood tests were repeated at 3-month intervals, ultrasound examinations were repeated at 6-month intervals.

The primary endpoint of the study was to evaluate patient survival in the compared groups: those receiving EBL and those receiving EBL plus NSBB.

NSBB was administered under control of heart rate and blood pressure, adjusting the dose when these parameters decreased. The initiating propranolol dose was 40 mg/day, the maximum dose was 240 mg/day. Carvedilol was started with 6.25 mg/day initiating dose, the maximum dose was 25 mg/day. Nadolol was started at 40 mg/day, with a maximum dose of 80 mg/day.

EBL was performed under sedation via esophagogastroduodenoscopy and band ligation of EV. Each EV was ligated with one or two latex ligatures (rings). Esophageal variceal ligation began at the gastroesophageal junction and continued proximally. As a rule, EV ligation was performed with 2 to 4 rubber ligatures or more, depending on the size of the EV. All patients underwent repeated procedures 4 weeks later until all EV meeting the WNT criteria [21] were obliterated. After EV obliteration, control esophagogastroduodenoscopy was performed at 3-month intervals. If a recurrence developed (a new EV appeared), repeated ligation procedures were performed.

All patients received diuretics, in some patients, in case of development of resistance to therapy, paracentesis was performed.

Statistical analysis of the data was carried out using the IBM SPSS Statistics program version 23. The Kolomogorov–Smirnov test was used to check the normal distribution of the indicators obtained during the study. Sample data with a normal distribution of the received data were presented as arithmetic means (M) and standard deviation (SD, standard deviation) with a 95% confidence interval (CI) determined. The statistical significance of differences between the compared values in the case of a normal distribution was determined by Student's t-test. In the absence of a normal distribution of obtained values of the studied indicators, the following nonparametric tests were used: Wilcoxon for paired comparisons of dependent variables, Mann–Whitney U-test,

Pearson's chi-squared test – for comparisons of independent variables. Quantitative indicators in samples with non-normal distributions were expressed as median and interquartile range (IQR, the interval between the 25th and 75th percentiles). For qualitative data, frequencies and fractions (%) were calculated. Differences between compared parameters were considered statistically significant if the probability of error was less than 0.05 ($p < 0.05$). Patient survival in the compared groups (EBL and EBL + NSBB) was determined by the Kaplan–Meier estimate; the log-rank (Mantel-Cox) test was used to compare survival. Predictors of waitlist mortality in the compared groups was also performed using the Cox proportional-hazards model with calculation of the hazard ratio (HR).

RESULTS

The mean waitlist follow-up was 46.8 months with ICR (1.4–65.2 months). A total of 86 patients with a mean age of 48.6 ± 13.1 years were included in the study, including 68 men (80%) and 18 women (20%). Table 1 and Table 2 present demographic, clinical, laboratory, and index data (MELD-Na, Child–Turcotte–Pugh) in the groups of patients with ascites who underwent EBL (n = 41) and EBL + NSBB (n = 45) for VB prevention during their stay in the LTWL. Of the 86 patients, 21 (24.4%) had no VB before being waitlisted, and 65 (75.6%) patients had VB before inclusion in the liver transplant waiting list.

There were no statistically significant differences in the structure of cirrhosis etiology (viral, non-viral). In both groups, patients had severe liver dysfunction as assessed by MELD index and Child–Turcotte–Pugh class of cirrhosis without significant differences between the compared groups. Grade 2 ascites predominated in both groups without statistically significant differences between the groups; the proportion of grade 3 ascites was also comparable in the compared groups (19.5% and 17.8%, respectively, $p > 0.05$). Of the 86 patients, the vast majority were on diuretics (83 patients, 96.5%). In the EBL group, 40 patients (97.6%) took diuretics; in the EBL + NSBB group, 43 patients (95.6%) did. There were no significant differences in the frequency of diuretics between the compared groups ($p < 0.05$). Intermittent paracentesis was performed against the background of diuretics in diuretic-resistant patients. There were no significant differences in RA incidence in the compared groups (14.6% and 17.7%, respectively, $p > 0.05$).

Table 1

Comparative characteristics of parameters of EBL and EBL + NSBB patients (normal distribution)

Indicator	EBL (n = 41) M ± SD	EBL + NSBB (n = 45) M ± SD	Significance of difference
Age	47.49 ± 11.16	49.59 ± 12.35	NS
Hemoglobin, g/L	113.43 ± 23.38	112.55 ± 25.61	NS
White blood cells $\times 10^9$/L	3.12 ± 0.43	3.07 ± 0.76	NS
Platelets, $\times 10^9$/L	98.39 ± 31.43	102.12 ± 35.43	NS
Plasma albumin, g/L	36.23 ± 4.54	34.74 ± 7.42	NS
MELD-Na	24.43 ± 4.35	25.45 ± 8.44	NS

Note: NS (non-significant), no statistically significant difference ($p > 0.05$) between compared values.

Table 2

Comparative characteristics of parameters of EBL and EBL + NSBB patients (no normal distribution)

Indicator	EBL (n = 41) Median (IQR) or [%]	EBL + NSBB (n = 45) Median (IQR) or [%]	Significance of difference
Male	32 (78.05%)	36 (80%)	NS
Viral etiology of cirrhosis	17 (41.5%)	21 (46.7%)	NS
Nonviral etiology of cirrhosis	24 (58.5%)	24 (53.3%)	NS
Ascites, grade 2	33 (80.5%)	37 (82.2%)	NS
Ascites, grade 3	8 (19.5%)	8 (17.8%)	NS
Varices, grade 2 (VNT)	21 (51.2%)	24 (53.3%)	NS
Varices, grade 3 (VNT)	20 (48.8%)	21 (46.7%)	NS
Child–Turcotte–Pugh, class B	23 (56.1%)	25 (55.6%)	NS
Child–Turcotte–Pugh, class C	18 (43.9%)	20 (44.4%)	NS
INR	2.05 (1.625–2.775)	1.95 (1.7–2.05)	NS
Creatinine, μmol/L	142.0 (110.0–198.25)	148.0 (111.5–202.5)	NS
Bilirubin, μmol/L	91.0 (67.25–206.5)	89.0 (62.5–987.5)	NS
Na, mmol/L	139.5 (138.0–141.0)	137.5 (135.5–143.5)	NS

Note: NS (non-significant), no statistically significant difference between compared values.

The compared groups had no statistically significant differences in the incidence of EV classified as VNT (NS).

No significant differences were found in the compared groups (NS) in terms of demographic, laboratory parameters.

During the stay in the LTWL, 39 patients died – 11 in the EBL group and 28 in the EBL + NSBB group. Table 3 shows the overall mortality, VB-associated mortality, liver dysfunction-associated mortality, and mortality due to other causes, as well as clinical outcomes (complications) developed during the therapy in the compared groups. As shown in Table 3, overall mortality was significantly higher in the EBL + NSBB group than in the EBL group. The VB-associated mortality, as well as liver dysfunction-associated mortality had no significant differences between the compared groups. At the same time, mortality associated with causes other than variceal bleeding or liver failure – portal vein thrombosis and renal dysfunction – was significantly higher in the group of patients receiving combined therapy than in the group of patients treated with EBL alone. There were no significant differences in the incidence of bleeding and spontaneous bacterial peritonitis against the background of the therapy in both compared groups. AKI developed more frequently in the group of patients treated with combination therapy (EBL + NSBB) than in the group treated with EBL alone.

Patient survival as determined by the Kaplan–Meier estimate (Fig. 1) was significantly higher in the EBL-treated group than in the EBL + NSBB group (log-rank = 0.001). The risk of death (Fig. 2) was significantly higher in the combination therapy (EBL + NSBB) group than in the EBL group (HR = 5.139; p = 0.005).

DISCUSSION

Cirrhosis is known to be the final stage attained by chronic liver diseases and it is the main cause of patient death regardless of its etiology. To date, two quite distinct stages of cirrhosis have been clearly formulated: compensation and decompensation, with different prognosis and pathophysiological mechanisms [32]. Compensated cirrhosis is a long-term asymptomatic stage, with average patient survival of over 12 years, while decompensated cirrhosis, whose main pathophysiological driver is CSPH, leads to VB, ascites, hepatic encephalopathy with a sharp decrease in patient survival – less than 2 years [23, 32].

Our study included patients with decompensated cirrhosis with the presence of ascites, risk of bleeding or VB.

It is known that ascites progression and bleeding are the leading causes of death in LTWL patients [7]. According to the updated Baveno VI guidelines, prevention of progression of decompensated cirrhosis and development of the first bleeding includes use of NSBB or EBL (primary prophylaxis) in patients with ascites, large varices (VNT), or with Child–Turcotte–Pugh classification class C. In order to prevent recurrent VB (secon-

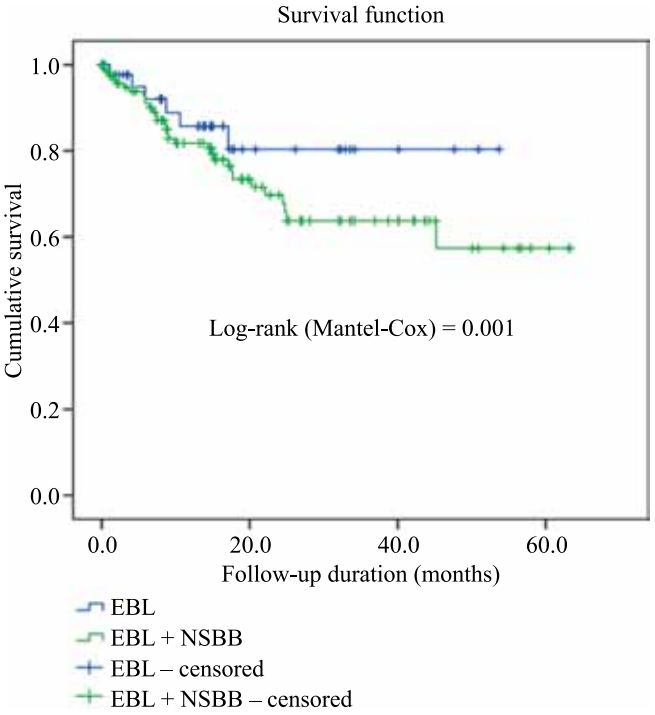

Fig. 1. Patient survival using Kaplan–Meier method with log-rank (Mantel-Cox) test in the EBL and EBL + NSBB groups

Table 3

Comparison of mortality and other clinical outcomes in EBL and EBL + NSBB patients

Indicator	EBL (n = 41) [%]	EBL + NSBB (n = 45) [%]	Significance of difference
Overall mortality	11 (26.8%)	16 (62.2%)	p = 0.001
Mortality associated with bleeding	3 (27.3%)	4 (25.0%)	NS
Mortality associated with liver failure	7 (63.6%)	9 (56.25%)	NS
Mortality associated with other causes	1 (9.1%)	3 (18.75%)	p = 0.002
Variceal bleeding	8 (19.5%)	10 (22.2%)	NS
Spontaneous bacterial peritonitis	2 (4.9 %)	3 (6.7%)	NS
Acute kidney injury	4 (9.75%)	9 (20%)	p = 0.031

Note: NS (non-significant), no statistically significant difference between compared values.

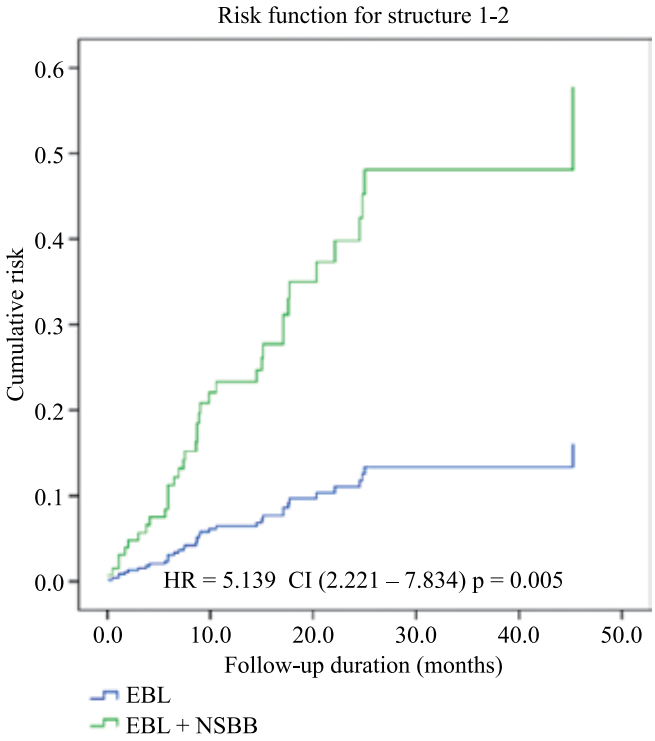

Fig. 2. Mortality in EBL and EBL + NSBB groups. Cox proportional hazards model with calculation of the Hazard Ratio (HR)

dary prevention), this consensus recommends the use of first-line therapy (combination of NSBB and EBL) [33].

In accordance with these guidelines, we used the NSBB + EBL combination predominantly for secondary prevention of variceal bleeding, and EBL for primary prevention. Although Baveno VI does not recommend the NSBB + EBL combination for primary prevention of VB, and EBL as an independent method of secondary bleeding prevention, these strategies are used in clinical practice [34]. In this regard, EBL procedure was used in a part of patients (about 30%) with a history of bleeding before inclusion in the LTWL, while the NSBB + EBL combined therapy was used in patients with no bleeding (35%).

Our analysis showed that both methods effectively prevented bleeding, achieving the objectives of primary and secondary prevention, as evidenced by the low incidence of VB and associated patient mortality against the background of the therapy; and there were no significant differences in bleeding incidence in the compared patient groups.

Nevertheless, we noted significant differences when assessing the overall patient mortality and survival in the compared groups. Overall mortality was significantly higher and patient survival was significantly lower in the EBL + NSBB group than in the EBL group. Similar results were obtained when analyzing mortality associated with the development of portal vein thrombosis and AKI. The EBL + NSBB patients were significantly more likely to develop AKI than their EBL counterparts.

How can these discouraging results in our research be explained? Indeed, propranolol, nadolol, and carvedilol have been shown to be useful agents in randomized clinical trials when used in patients with ascites and VNT, being a first-line therapy in the prevention of VB [33, 35]. However, in the above studies, patients with severe and, especially, refractory ascites, who are highly likely to develop AKI, were excluded from calculations [36]. Accordingly, even the updated guidelines for management of patients with ascites and the risk of VB [33, 36] cannot be automatically extrapolated to patients with severe decompensated cirrhosis with significant hemodynamic disorders [37, 38]. This is probably confirmed by our data indicating an increase in mortality in patients with ascites who received combined therapy (EBL + NSBB), as well as an increase in patients with AKI in the same group of patients. Undoubtedly, decreased patient survival and increased risk of mortality obtained for this patient cohort are also associated with the adverse effects of NSBB on hemodynamics.

Three pathophysiological mechanisms may explain the negative impact of NSBB on ascites patients at high risk for VB. First, in at least some patients with ascites, the cause of high mortality is a decrease in mean arterial pressure (SBP, MAP in the English literature). MAP develops during all phases of the cardiac cycle, is the product of CO and total peripheral resistance (OPS), to which is added the value of the central venous pressure (CVP). It has been shown that in a large cohort of waitlisted patients with ascites, NSBB significantly reduced patient survival due to a decrease in MAP <80 mm Hg [39]. Secondly, NSBB, by inhibiting the increase in compensatory CO in response to increased vasodilation, leads to a significant decrease in the survival of patients with cirrhosis and RA [40]. Thirdly, NSBB through β-adrenergic receptor blockade is associated with higher risk of kidney damage (AKI and hepatorenal syndrome (HRS)) in patients with severe decompensated cirrhosis (Child–Turcotte–Pugh class C). Thus, the risk of developing HRS and AKI was three times higher in patients with ascites who received NSBB compared to patients who did not receive these drugs [41]. In cirrhosis patients with ascites, who are included in the LTWL, the risk of developing post-NSBB AKI was increased by more than three times compared with patients without ascites, in whom the use of these drugs was associated with an 80% reduction in AKI incidence [42].

It should be noted that a number of foreign researchers have shown results similar to ours. For example, Jeong-Ju Yoo et al. [43] found a significant decrease in survival and an increase in overall mortality in patients who received combined propranolol and EBL therapy compared to patients who received EBL only.

CONCLUSION

Our studies have shown that both methods (EBL and EBL + NSBB) performed for primary or secondary prevention of VB, effectively reduce VB incidence. However, the presence of ascites, and especially RA, significantly increases mortality in patients treated with the EBL + NSBB combination. Reduced patient survival in this group is probably due to the negative impact of NSBB on cardiovascular haemodynamics at this stage of PH progression (reduced SBP, reduced CO), which in turn results in reduced renal perfusion and a significant increase in AKI. In order to improve patient survival, it is necessary to differentiate the use of different representatives of this class depending on the cardiac hemodynamics parameters and to apply the NSBB dose titration principle.

The authors declare no conflict of interest.

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EXPERIENCE IN THE USE OF INVASIVE HEMODYNAMIC MONITORING USING PREPULMONARY AND TRANSPULMONARY THERMODILUTION IN LUNG TRANSPLANTATION

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Objective: to demonstrate the experience of using complex hemodynamic monitoring by means of prepulmonary thermodilution (PPTD) and transpulmonary thermodilution (TPTD) – PiCCO – in lung transplantation (LTx). **Materials and methods.** Presented is a clinical case study of a 51-year-old patient with the following diagnosis: severe bronchiectasis and type 3 respiratory failure. Bilateral lung transplantation was performed at Sklifosovsky Research Institute for Emergency Medicine, Moscow. Intraoperative hemodynamic monitoring was performed using PPTD and TPTD techniques. **Conclusion.** The case study presented shows that simultaneous use of PPTD and TPTD for hemodynamic monitoring during lung transplantation achieves better treatment outcomes. This hemodynamics monitoring strategy is highly informative, allows for continuous measurement of necessary hemodynamic parameters and for timely and targeted correction of identified disorders by influencing the basic pathogenesis links of cardiovascular disease.

Keywords: lung transplantation, hemodynamic monitoring, transpulmonary thermodilution, PiCCO, prepulmonary thermodilution, intraoperative period.

BACKGROUND

Bilateral LTx is the only radical method for treating end-stage lung diseases [1, 2]. This type of surgical intervention often comes with hemodynamic instability at different stages, including during anesthesia induction, pulmonary artery clamping, after reperfusion and during implanted graft ventilation. Therefore, comprehensive continuous hemodynamic monitoring is necessary. Control of systemic and pulmonary hemodynamics is crucial for intraoperative management of this condition [1, 3, 4]. Adequate invasive hemodynamic monitoring allows for targeted correction of arising disorders by changing the infusion therapy tactics, using inotropic and vasopressor drugs, etc. [5].

Currently, there are no clinical guidelines for intraoperative hemodynamic monitoring in lung transplantation [1]. The main methods in this role are: invasive monitoring of blood pressure (BP), central venous pressure (CVP), PPTD using pulmonary artery catheterization (PAC), TPTD, transesophageal echocardiogram. TPTD allows a number of important hemodynamic parameters to be recorded: cardiac output (CO), CVP, pulmonary artery pressure (PAP), pulmonary artery occlusion pressure (PAOP), right atrial pressure, etc. [1, 6, 7].

The introduction of TPTD into clinical practice has made it possible to expand hemodynamic monitoring. The advantage of TPTD over PPTD is the measurement of a number of additional parameters, such as intrathoracic blood volume (ITBV), global end-diastolic volume (GEDV), extravascular lung water index (EVLWI), indicating the volumic status of the patient. TPTD method is less invasive and technically easier than PAC, the values obtained by TPTD more accurately reflect the formation of pulmonary edema, ahead of changes in gas exchange [8]. Volumetric monitoring by TPTD method is relevant in any critical conditions accompanied by impaired heart pumping function, increased permeability, gas exchange disorders, "capillary leakage" and tissue hypoperfusion, including patients undergoing LTx [3, 5].

Transpulmonary thermodilution method has been widely used in clinical practice with the advent of modern hemodynamic monitors PiCCO (Pulsion, Germany) [3, 6]. PiCCO monitoring technology combines two methods: transpulmonary hemodilution and arterial pulse wave analysis. It provides an assessment of volumetric preload, contractility, afterload indices, extravascular lung water volume, and cardiovascular response to volume load [5].

Simultaneous use of PPTD and TPTD makes it possible to obtain the results of measuring not only the pressures but also the volumes of all right and left heart chambers [6].

The **objective of this work** was to demonstrate the experience of comprehensive hemodynamic monitoring using PPTD and TPTD (PiCCO) in lung transplantation.

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CASE STUDY

Patient S., 51 years old, diagnosed with severe bronchiectasis. Bilateral LTx was performed at Sklifosovsky Research Institute for Emergency Medicine in Moscow.

Before induction of anesthesia, 100% oxygen was preoxygenated with an anesthesia mask. Fentanyl (3–5 μg/kg) and propofol (1.5–2 μg/kg) were used to induce anesthesia, and rocuronium bromide 1 mg/kg was applied for myorelaxation. After induction of anesthesia, a 39 Fr (Left Broncho-Cath; Mallinckrodt, Athlone, Ireland) double-lumen endobronchial tube was placed. Intraoperative artificial ventilation was performed using Drager Primus (Germany) in volume control mode (VCV) with 500–600 ml respiratory volume and 12–14 per minute respiratory rate with short inspiratory time and maximum expiratory time, maintaining peak inspiratory pressure <35 cm H_2O with oxygen fraction from 0.6 to 1.0. Positive end-expiratory pressure was 4 to 5 cm H_2O. Anesthesia was maintained with sevoflurane (0.5 MAC) and continuous infusion of fentanyl (2–4 μg/kg/hr). A warming blanket (Gamar Meditherm, Orchard Park, NY) was used to control body temperature.

Intraoperative monitoring included electrocardiography, pulse oximetry, capnometry, noninvasive and invasive blood pressure (BP), PPTD and TPTD using Dräger Infinity Delta XL+ PiCCO Dräger attachment system, Germany. A Dräger cardiac monitor (Germany) was used. For invasive BP monitoring after induction of anesthesia, we placed a catheter in the left radial artery 20 G (B. braun Germany). We also performed left subclavian vein catheterization with a 12 Fr three-lumen high-flow central venous catheter (B. braun, Germany) and inserted an 8.5 Fr introducer (Baxter Edwards Laboratories) into the right internal jugular vein to insert a Swan–Ganz catheter (F131HF7; Edwards LifeSciences, USA). A 5 F arterial catheter (Pulsiocath PV2015L20; Pulsion Medical System) was inserted into the left common femoral artery. Pressures in different parts of the vascular bed and/or heart chambers were measured with the reference point being the midaxillary line level and the fourth intercostal space plane, with the patient in a strictly horizontal position using a monitor. We also assessed acid-base status and water-electrolyte balance, determined SvO_2 and lactate using a Radiometer ABL800 Flex gas analyzer (Denmark). The patient did not require extracorporeal membrane oxygenation (ECMO) in the intraoperative period.

The patient was given an intravenous infusion of Sterofundin solution to replenish the initial volume. In case of a <3 decrease in cardiac index (CI), 5 to 8 mcg/kg/min dobutamine was administered against the background of infusion therapy. In hypotension with BP <60 mm Hg, 0.02–5 μg/kg/min norepinephrine was administered. Fresh frozen plasma was administered if INR >2. Red blood cell mass was transfused to maintain hemoglobin levels >9 g/dL. Intraoperative blood loss was recorded by measuring the volume of collected blood in a cell saver.

Central hemodynamic parameters – PAP, PAOP, CI, EVLWI, global end-diastolic volume index (GEDVI), ITBV, stroke volume index (SVI), systemic vascular resistance index (SVRI), etc. – were recorded at the following stages: after anesthesia induction, after left pneumonectomy, after left lung reperfusion, after right lung pneumonectomy, after right lung reperfusion, and after chest closure. Hemodynamic monitoring results are presented in Table.

Data analysis showed that initially, the patient's mPAP and PAOP were elevated relative to baseline after induction of anesthesia. After left pneumonectomy, there was increased CVP, mPAP and PAOP. Meanwhile, EVLWI remained at the same level, ITBVI slightly decreased. At the stage of pneumonectomy, during pulmonary artery clamping, there was a 2.7 l/min/m^2 decrease in CI, which required inotropic support (5 μg/kg/min dobutamine); CI was 3.4 l/min/m^2. After left lung reperfusion, a 1000 dyn·s·cm decrease in SVRI against the background of reperfusion syndrome, 15 ml/m^2 increase in EVLWI and 1145 ml/m^2 increase in ITBVI were detected. The clinical decision at this stage was to administer norepinephrine 0.2 mcg/kg/min and restrict infusion therapy and conduct dehydration (furosemide). The data obtained at the stage of right lung pneumonectomy showed a decrease in ITBVI and EVLWI, and an increase in SVRI. Due to the increase in EVLWI and ITBVI during right lung reperfusion, a decision was made to limit infusion therapy and perform dehydration (furosemide). This tactic allowed to correct the abnormalities. The obtained data indicated that CI and CAPWA values were consistent when measured by two methods. The volume of intraoperative infusion-transfusion therapy was 6200 mL, blood loss 1200 mL, urine volume 1900 mL, and perspiration volume 400 mL. The total balance was +2700 mL.

DISCUSSION

Central hemodynamic monitoring is necessary in LTx due to high likelihood of hemodynamic instability episodes and the need for their prompt correction [3, 7, 8]. Until now, there is no consensus on a hemodynamic monitoring method. This has created uncertainty in the choice of a particular method in this type of surgical intervention. In our opinion, this is due to the presence of a wide range of invasive and noninvasive techniques implemented in various hemodynamic monitoring devices, and the uniqueness of LTx as a surgical intervention, which consists in successively changing stages, accompanied by hemodynamic instability. According to a multicenter cross-sectional study evaluating the frequency of hemodynamic monitoring methods in LTx in different centers, PPTD is used in 69% of cases, while

PPTD and TPTD are used together in 17.8% of cases. Transesophageal echocardiography is used in most cases (89.3%) [7].

According to the literature, methods currently used in LTx have not been validated and each of them has limitations and shortcomings. Transesophageal echocardiogram is a widely used hemodynamic monitoring method [3]. Despite rapid diagnosis of hemodynamic instability using this method, it should be noted that it is an intermittent method, highly operator-dependent, and peak pulmonary venous flow velocities can be overestimated [7]. Operator dependence and high cost limit the use of transesophageal echocardiography in clinical practice, although its use in LTx is recommended in most countries.

The PPTD method is based on the StewartSwan–GanzHamilton principle, which describes the dilution of the indicator and requires a Swan–Ganz catheter equipped with a thermistor [6]. The obtained indicators are of clinical significance in cases complicated for pathogenetic interpretation. PPTD monitoring does not always fully reflect the volemic and hemodynamic status of a patient. According to Rocca et al., PAOP is not a reliable indicator for cardiac preload measurement, which is crucial for volumetric therapy and administration of inotropes and vasopressors [3].

The most comprehensive measurement of intraoperative hemodynamic parameters in LTx is possible with TPTD. In recent years, due to acceptable accuracy, less invasiveness and possibility of volumetric monitoring, TPTD has practically replaced the prepulmonary technique. The study conducted by Rocca et al. showed that ITBV is a more reliable indicator of cardiac preload compared to PAOP in LTx [3]. Similar results have been arrived at by Brock H. [9]. A number of researchers have demonstrated that such indicators as GEDVI and EVLWI in LTx allow predicting the development of primary graft dysfunction (PGD) and adjusting the treatment tactics accordingly in time [8, 10, 11]. Hofer C.K. showed that there is a close correlation between the data obtained by TPTD measurement and transesophageal echocardiography [12].

It should be noted that the use of PPTD and TPTD in the intraoperative period of LTx in case of ECMO has been questioned by a number of researchers because of the release of the indicator into the extracorporeal circuit at high flow [13, 14]. The need for venoarterial (VA) ECMO and venovenous (VV) ECMO in this surgery is often due to the need for hemodynamic support, correction of pulmonary gas exchange, and restoration of systemic perfusion, which in turn provides reperfusion and protective ventilation of the graft, thereby reducing ischemia-reperfusion injury [2, 15, 16, 17]. A study by Herner et al. demonstrated that both GEDVI and EVLWI are overestimated during TPTD in patients undergoing VV ECMO, but hemodynamic parameters such as CO, SV, SVI, CI, etc were not affected [13].

This clinical case study has demonstrated that simultaneous use of PPTD and TPTD in the intraoperative period during LTx is the optimal way for hemodynamic monitoring because of the wider range of parameters obtained.

Table

Results of PPTD and PiCCO monitoring at different stages of surgery

Indicators	After anesthesia induction	After left pneumonectomy	After left lung reperfusion	After right lung pneumonectomy	After right lung reperfusion	After chest suturing
BP avr., mm Hg	72	75	65	75	68	79
CVP, mm Hg	12	16	11	13	10	10
HR, bpm	88	98	100	107	110	99
PAOP, mm Hg (N = 6–12)	19	22	20	22	15	14
mPAP, mm Hg (N = 17–23)	51	59	35	43	26	25
CI, L/min/m^2 (N = 3–5)	3.4	3.3	3.4	3	3.4	3.4
ITBVI, mL/m^2 (N = 850–1000)	928	860	1145	956	1532	1100
SVV, % (N = to 10%)	12	10	8	7	5	7
SVRI, dyn·s·cm^{-5} (N = 1200–2200)	1411	1522	1270	1653	1325	1623
CCI_{art}, L/min/m^2 (N = 3–5)	3.4	3.4	3.3	3	3.5	3.4
SV, mL (N = 50–120)	65	53	56	50	59	57
EVLWI, mL/m^2 (N = 3–7)	7	8	15	12	20	9

Note: BP, blood pressure; CVP, central venous pressure; HR, heart rate; PAOP, pulmonary artery occlusion pressure; mPAP, mean pulmonary artery pressure; ITBV, intrathoracic blood volume; ITBVI, intrathoracic blood volume index; SVV, stroke volume variation; SVRI, systemic vascular resistance index; CCI_{art}, continuous cardiac index by arterial waveform analysis (PiCCO); CI, cardiac index (prepulmonary thermodilution using Swan–Ganz catheter); SV, stroke volume; EVLWI, extravascular lung water index.

CONCLUSION

The presented case study shows that simultaneous use of PPTD and TPTD for hemodynamic monitoring during lung transplantation achieves better treatment outcomes. This hemodynamics monitoring strategy is highly informative, allows for continuous measurement of necessary hemodynamic parameters and for timely and targeted correction of identified disorders by influencing the basic pathogenesis links of cardiovascular disease.

The authors declare no conflict of interest.

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EARLY DIAGNOSIS AND TREATMENT OF SPLENIC ARTERY STEAL SYNDROME AFTER LIVER TRANSPLANTATION

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Objective: to study the incidence of splenic artery steal syndrome (SASS) in our own series of liver transplant surgeries and to determine diagnostic and therapeutic tactics. **Materials and Methods.** During the 3.5 years of existence of the liver transplant program in the Republic of Tatarstan, 77 cadaveric liver transplantations (LTx) have been performed. Postoperative SASS occurred in 4 cases (5.2%). Among the patients were 3 women and 1 man; mean age was 38 years. Doppler ultrasonography of the liver vessels and celiacography were used for diagnosis. Proximal splenic embolization was used as a way to correct the syndrome. **Results.** In all clinical cases, SASS was timely diagnosed and corrected by endovascular image-guided intervention. The patients were discharged with good hepatic graft function. The complication did not affect the length of hospital stay. **Conclusion.** SASS remains a severe vascular complication of LTx, which can lead to graft dysfunction and possible loss. Timely detection and treatment prevent severe consequences for the liver recipient.

Keywords: liver transplantation, splenic artery steal syndrome, splenic embolization.

INTRODUCTION

LTx remains the only radical treatment for end-stage liver diseases. However, such high-tech interventions come with specific vascular complications. These complications can be initiated both by primary problems in the area of venous and arterial anastomoses, and by changes in hepatic hemodynamics caused by postoperative cirrhosis. Insufficient arterial or excessive portal perfusion of the graft can lead to severe consequences, even to graft loss.

SASS is still not a well understood vascular complication following LTx. The reported incidence of SASS in LTx recipients ranges from 0.6% to 10.1% [1]. It is characterized by decreased blood flow through the hepatic artery in the absence of occlusive disease of the hepatic artery, associated with increased blood flow through the dilated splenic artery or more rarely through the gastroduodenal artery [2]. Hypoperfusion develops in the graft, which can lead to severe ischemic injury of the organ up to the need for retransplantation [3, 4].

There are no clear ideas about the pathogenesis of SASS and its diagnostic criteria that allow for early prevention of graft dysfunction by timely image-guided surgery. In this paper, we want to demonstrate SASS cases with active treatment tactics.

MATERIAL AND METHODS

From December 2018 to May 2022, 77 cadaveric liver transplants were performed at the second surgical ward of Republican Clinical Hospital, Kazan. Postoperative SASS occurred in 4 cases (5.2%). All patients who developed this syndrome were operated on using the same technique (J. Belghiti's side-to-side cavo-caval anastomosis, end-to-end portal anastomosis, arterial reconstruction using the recipient's gastroduodenal artery, and end-to-end biliary anastomosis). Among them were three women and one man; the mean age was 38 years. Evaluated were the indicators of X-ray computed tomography (CT) performed in patients with up to one month before LTx – splenic and hepatic artery diameters, variant anatomy of branches of the celiac trunk and superior mesenteric artery.

After LTx, we used Doppler ultrasonography of hepatic vessels as a screening – twice a day during the first week after surgery, then when indicated and before discharge. General clinical and biochemical laboratory indicators were studied (twice a day during the stay in the intensive care unit, daily for 3–5 days after transfer to the ward, then when indicated and before discharge). The tables below mainly show the data on the day of SASS detection, as well as on days 1, 3, 5, and 10 after image-guided correction of this complication. The final diagnosis of splenic steal syndrome was established by celiacography, which in all cases revealed depleted blood flow with late filling of the hepatic artery with preferential blood flow through the splenic artery. Control celiacography immediately after splenic artery occlusion in the proximal part showed increased blood flow in the hepatic artery with improved blood supply

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to the peripheral parts of the liver parenchyma. Below we describe clinical case.

CLINICAL CASE 1

Male patient A., 34 years old, diagnosed with liver cirrhosis that resulted from primary sclerosing cholangitis, Child–Pugh class B, esophageal varices grade 3 complicated by repeated bleeding, ascites, bilateral hydrothorax, splenomegaly. MELD score 13. According to X-ray CT conducted on March 19, 2022, the spleen size was 15.5 × 15 × 7.3 cm, diameters of the splenic artery and hepatic artery were 7.5 mm and 5.5 mm, respectively. Transjugular intrahepatic portosystemic shunt was performed on March 23, 2022. Orthotopic LTx (OLTx) was carried out on March 26, 2022 due to availability of a compatible deceased donor. On day 1 after surgery (March 27, 2022), SASS was suspected via Doppler ultrasonography, which was confirmed by celiacography (Fig. 1). The splenic artery was embolized proximally for SASS (Fig. 2). Control celiacography after embolization showed good contrasting of the hepatic artery and its distal bed (Fig. 3). The postoperative period further proceeded smoothly; the patient was discharged on day 17. Laboratory and instrumental data are presented in Table 1.

CLINICAL CASE 2

Female patient A., 33 years old, presented with liver cirrhosis that resulted from autoimmune hepatitis; Child–Pugh class C, varices grade 3, three times complicated by bleeding, ascites, bilateral hydrothorax. MELD score 17.

According to X-ray CT conducted on February 30, 2022, the spleen dimensions were within normal values, the diameter of the splenic artery was 7.5 mm, that of the hepatic artery was 7.8 mm. Transjugular intrahepatic portosystemic shunt was performed on March 11, 2022. OLTx was performed on March 30, 2022. On day 8 after the surgical intervention, there was decreased peak systolic hepatic artery flow velocity with no diastolic velocity. At the same time, splenic artery velocity increased. On day 9 after the operation, due to deterioration in Doppler ultrasonography of the liver vessels, celiac trunk angiography was performed, in which the splenic embolization syndrome was confirmed, and proximal splenic embolization was performed. Control angiography showed good filling of the hepatic artery. Biochemical parameters and ultrasound data are presented in Table 2. The postoperative period further proceeded smoothly; the patient was discharged on day 14.

CLINICAL CASE 3

Female patient C., 27 years old; on April 19, 2022 underwent OLTx for cirrhosis that resulted from autoim-

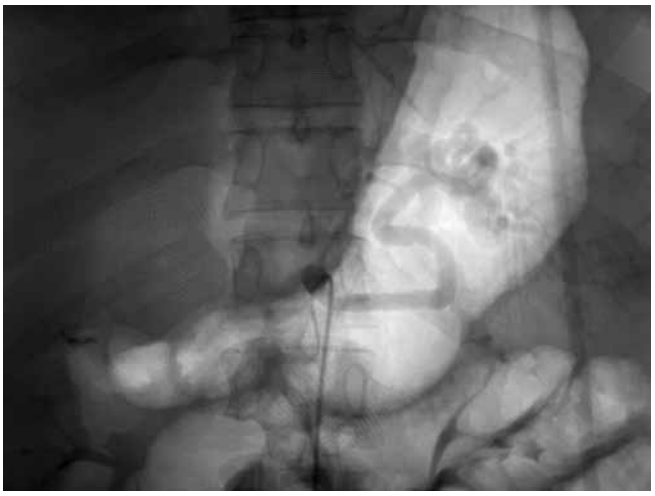

Fig. 1. Celiacography. The splenic artery is contrasted, the hepatic artery and its distal bed are not contrasted

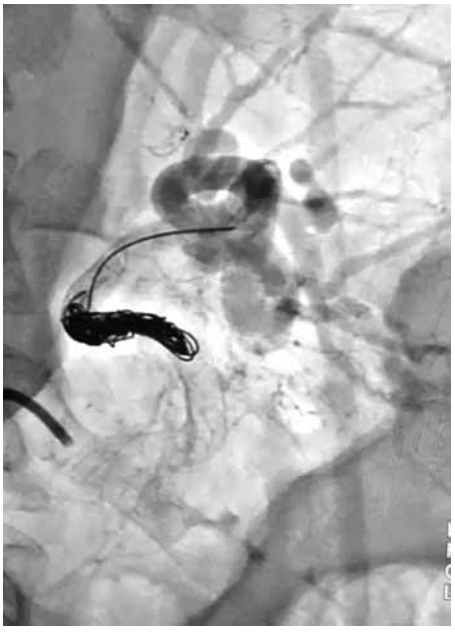

Fig. 2. Proximal splenic embolization using stent and coils

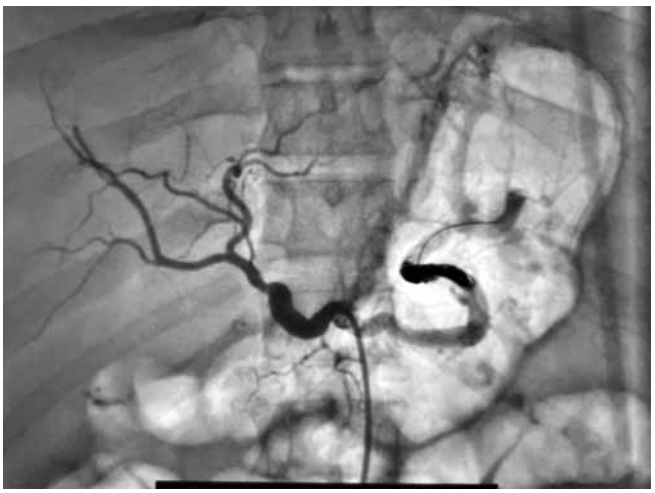

Fig. 3. Control angiography showing good filling of the hepatic artery

mune hepatitis; Child–Pugh class B, esophageal varices grade 1, ascites, grade 1 recurrent hepatic encephalopathy. MELD score 16.

According to X-ray CT conducted on April 19, 2022, the spleen size was 16 × 6 × 12 cm, diameters of the splenic artery and hepatic artery were 7 mm and 3 mm, respectively. On day 1 after surgery (April 20, 2022), SASS was suspected via Doppler ultrasonography, which was confirmed by celiacography. Proximal splenic embolization was performed. Control angiography showed good filling of the hepatic artery. The postoperative period further proceeded smoothly; the patient was discharged on day 22. The dynamics of the parameters are presented in Table 3.

Table 1

Dynamics of biochemical parameters and ultrasound data (clinical case #1)

	26.03.22	27.03.22	28.03.22	30.03.22	01.04.22	06.04.22
Biochemical markers		embolization				
Alanine aminotransferase	30	1123	879.4	554	236	116.1
Aspartate aminotransferase	33	1023	388	134	28	17.3
Alkaline phosphatase	295	138	70.9	150	164	92
Gamma-glutamyl transferase	68	114	93.8	228	229	123
Total bilirubin	50.5	68.5	37.9	31.5	22.6	10.7
Doppler ultrasound data						
VP velocity		36.8	26	26	24	24
Vertebral artery peak systolic velocity	not visualized	24	20	63	29.9	72.9
Vertebral artery end-diastolic velocity		absence	absence	26	6.4	16.5
Vertebral artery resistance index				0.59	0.78	0.79
Carotid artery peak systolic velocity	65	135		48	40	75.7

Table 2

Dynamics of biochemical parameters and ultrasound data (clinical case #2)

	03.04.2022	06.04.2022	07.04.2022	08.04.2022	10.04.2022	12.04.2022
Biochemical markers			embolization			
Alanine aminotransferase	189.6	100	50.7	69	32.9	32.1
Aspartate aminotransferase	66.9	19	13.8	19	9.3	10.6
Alkaline phosphatase	179	139	67.6	123	88	86.4
Gamma-glutamyl transferase	596	434	228	351	273	269
Total bilirubin	58.9	27.1	12.9	27.1	22.7	25.32
Doppler ultrasound data						
VP velocity	58	33	26	32	31	
Vertebral artery peak systolic velocity	118	20	16.9	69.2	46.1	
Vertebral artery end-diastolic velocity	49.4	absence	4.4	28.2	24.9	
Vertebral artery resistance index	0.58		0.74	0.59	0.46	
Carotid artery peak systolic velocity	63	84.2	133	89.5	56.9	

Table 3

Dynamics of biochemical parameters and ultrasound data (clinical case #3)

	20.04.2022	21.04.2022	23.04.2022	25.04.2022	30.04.2022
Biochemical markers	embolization				
Alanine aminotransferase	265.3	228.6	303	264	60
Aspartate aminotransferase	477.6	192.1	161	111	15
Alkaline phosphatase	68.3	68	101	104	87
Gamma-glutamyl transferase	49.2	47	247	236	79
Total bilirubin	65	46.8	49.2	44.7	28
Doppler ultrasound data					
VP velocity	55	40	45	50	68
Vertebral artery peak systolic velocity	not visualized	22	32	35	61.2
Vertebral artery end-diastolic velocity			5.5	6	11.8
Vertebral artery resistance index			0.83	0.83	0.81
Carotid artery peak systolic velocity	103	52	85	57	104

CLINICAL CASE 4

Female patient A., 58 years old, on March 29, 2022 had OLTx for cirrhosis that resulted from overlap syndrome (primary biliary cirrhosis combined with autoimmune hepatitis, Child–Pugh class C, esophageal varices grades 2–3, ascites. MELD score 26. According to X-ray CT conducted on March 28, 2022, the spleen size was 13.5 × 8.5 × 5.8 cm, diameters of the splenic artery and hepatic artery were each 6 mm. On April 1, 2022, celiacography with subsequent splenic embolization was performed due to suspected SASS. Laboratory and instrumental data are presented in Table 4. The postoperative period further proceeded smoothly; the patient was discharged on day 24.

DISCUSSION

The SASS phenomenon was first described by Manner in 1991 [5]. The authors suggested that the delayed filling of the hepatic artery with contrast according to arteriography was associated with preferential outflow of blood into the dilated splenic artery in patients with severe splenomegaly. However, in 2008, Quintini C. et al. proposed an alternative theory of portal hyperperfusion [6]. According to their data, hepatic artery narrowing in these patients occurred in response to increased portal blood flow. Transplant hyperperfusion along the portal vein causes sinusoidal damage due to the direct effect of increased portal pressure on liver cells and due to hepatic artery buffer response (HABR). HABR allows adequate hepatic blood flow to be maintained by vasodilator adenosine. A decrease in portal flow washes away less adenosine, which accumulates to dilate the hepatic artery and increase arterial blood flow. In the case of SASS, increased portal venous blood flow accelerates adenosine washout, which causes relative vasoconstriction of the hepatic artery [7]. A clinical case with a rare anatomical anomaly supports this theory [8]. In a patient with SASS, there was an absence of the splenic trunk with the splenic artery branching separately from the common hepatic artery directly from the aorta, which excludes the very process of "stealing". In 2012, Saad W.E.A. et al. suggested that HABR is only one of the potential causes of SASS, along with splenic or gastroduodenal artery steal syndrome and proposed a new name – post-transplant nonocclusive hepatic artery hypoperfusion syndrome [9]. Thus, there are no studies, which would reliably determine the pathogenetic aspects of reduced blood flow along the hepatic artery in patients after LTx.

SASS is a diagnosis requiring the exclusion of other vascular complications (thrombosis, hepatic artery stenosis), graft rejection, and infections [4, 10]. The timing of SASS ranges from a few hours to 5.5 years after surgical intervention, but more often in the first 3 months [10]. The clinical picture is nonspecific, ranging from the absence of symptoms to manifestations of severe graft dysfunction. Biochemical findings may include hyperbilirubinemia, increased levels of transaminases, alkaline phosphatase, and gamma-glutamyltransferase [4, 10].

Ultrasound examination of hepatic vessels is the method of choice for screening of the pathology. According to studies, SASS is characterized by decreased hepatic artery blood flow velocity less than 35 cm/s, resistance index more than 0.8, low or reversed diastolic blood flow [11]. At the same time, there is increased velocity along the portal vein and splenic artery. Given the presence of splenomegaly in most patients with cirrhosis, the detection of enlarged spleen has no diagnostic significance for SASS verification.

The most reliable data for diagnosis of hepatic artery hypoperfusion can be obtained by computed tomographic angiography. Kirbas I. et al. reported that a splenic artery size ≥4 mm or >150% of the hepatic artery diameter was associated with SASS [12]. Such multidetector CT signs as splenic volume >829 mL, splenic

Table 4

Dynamics of biochemical parameters and ultrasound data (clinical case #4)

	30.03.22	31.03.22	01.04.22	02.04.22	04.04.22	06.04.22	11.04.22
Biochemical markers			embolization				
Alanine aminotransferase	382.3	217.2	187.4	150.9	109.2	121	43.1
Aspartate aminotransferase	428	134	65.9	40.9	43.3	31	19
Alkaline phosphatase	170	107	110	55.3	45.9	132	170
Gamma-glutamyl transferase	70	41	49	54	35.4	41	22.9
Total bilirubin	178.9	138.8	118.2	96.4	68.8	70.7	40.4
Doppler ultrasound data							
VP velocity	80	49	33	33	74	34	25
Vertebral artery peak systolic velocity	61	100	32	79	53.5	81	48
Vertebral artery end-diastolic velocity	15	14	absence	19.8	16.5	29	21
Vertebral artery resistance index	0.75	0.86		0.75	0.69	0.64	0.56
Carotid artery peak systolic velocity	52	86	116		80		79

artery diameter >4 mm and differences of 6 mm between splenic and hepatic artery diameters are described as preoperative predictors of SASS [12, 13, 14].

Slow and delayed blood flow in the hepatic artery, early perfusion of the splenic or gastroduodenal artery are the key angiographic findings. In severe cases, portal venous blood flow is contrasted simultaneously with splenic arterial blood flow or even before complete filling of the hepatic artery [4, 9].

The aim of SASS therapy is to increase blood flow in the hepatic artery. The preferred method is splenic embolization due to its minimal invasiveness and effectiveness. According to the literature, more proximal placement of coils preserves collateral blood flow to the spleen, thus reducing the risk of complications such as spleen infarction and sepsis [4, 10, 15]. However, Fleckenstein et al., in a comparison of laboratory parameters of 75 liver transplant recipients with SASS, revealed no reliable differences in long-term outcomes depending on the place of splenic embolization [16]. Thus, the place to embolize the splenic artery is left for the physician to decide. If interventional treatment is ineffective or impossible, surgical options – splenic artery ligation or splenectomy – are considered [10].

Splenic artery ligation during LTx in the presence of risk factors is used as SASS prevention.

In our study, all patients had a splenic artery dilation >4 mm according to CT scans before LTx. This is consistent with literature data on identification of SASS predictors.

It is generally accepted that SASS is associated with graft dysfunction manifested by elevated liver function values with or without clinical signs (ascites). However, early after liver transplantation, ischemia-reperfusion injury may mask the biochemical changes suggestive of SASS. Therefore, the main focus for screening of this syndrome should be Doppler ultrasonography of the liver.

In addition, our small experience shows that timely celiacography to verify SASS with endovascular image-guided proximal occlusion of the splenic artery helps to avoid ischemic manifestations and severe graft dysfunction. In the early postoperative period, there were no complications associated with splenic embolization; all patients were discharged with satisfactory graft function.

We consider the following SASS diagnostic algorithm to be optimal. If blood flow linear velocity along the hepatic artery is reduced and that of the splenic artery and portal vein is simultaneously increased according to Doppler ultrasound of hepatic vessels, the study is repeated after 6 hours. If the tendency to changes in blood flow persists, celiacography is performed. Proximal splenic embolization is performed if typical SASS angiographic signs are revealed.

CONCLUSION

Thus, SASS remains a severe vascular complication of LTx that can lead to graft dysfunction and possible loss. Timely detection and correction of SASS could prevent severe consequences for the liver recipient. The issue of prevention of this complication remains debatable, which undoubtedly requires further research in the study of visceral venous and arterial blood supply in cirrhosis and after LTx.

The authors declare no conflict of interest.

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PERIOPERATIVE PERIOD IN HEART TRANSPLANTATION WITH EXTREMELY PROLONGED ISCHEMIC TIMES (>6 HOURS)

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Amidst the shortage in viable donor hearts, the use of hearts from expanded criteria donors, including those with prolonged ischemic time, remains one of the real ways to increase the donor pool and number of heart transplantations (HTx) performed. The study included 38 recipients (33 (86.8%) men and 5 (13.2%) women) aged 11 to 66 (44.7 ± 12.0 years, median 48.0 years), who underwent primary (n = 37; 97.4%) or repeat (n = 1; 2.6%) HTx (retransplantation). Donor hearts (n = 38) with ischemic time ranged from 362 (6 hours 2 minutes) to 571 (9 hours 31 minutes) or 407 ± 52 minutes (median 400 minutes). In 33 (86.8%) of 38 recipients, the early posttransplant period was characterized by satisfactory initial graft function. Five (13.1%) recipients developed severe primary graft dysfunction, requiring post-transplant venoarterial extracorporeal membrane oxygenation (VA-ECMO) (n = 4; 10.5%) or prolongation of pre-transplant VA-ECMO within 8 days of HTx (n = 1; 2.6%). In-hospital mortality was 7.9% (n = 3). Thirty-five (92.1%) of 38 recipients were discharged from the hospital. Three recipients died in the post-hospital period at day 734, 944, and 2146 after HTx. Thirty-two (84.2%) of the 38 recipients remained alive at the end of the study. Our own experience shows that HTx from donors with prolonged ischemic time could be effective.

Keywords: heart transplantation, prolonged ischemic time.

INTRODUCTION

In the context of shortage of viable donor hearts, the use of hearts from expanded criteria donors remains one of the real ways to increase the donor pool and the number of HT performed [1, 2, 3]. Suspected prolonged (>4 hours) donor ischemic time due to the time it takes to transport the donor heart to a transplant center (transport ischemia) or other reasons is one of the leading factors of expanded heart donation [4]. Despite the existing concerns on a more frequent severe primary dysfunction, HTx with prolonged ischemic time continues to be performed and is considered as one of the measures to eliminate donor organ shortage and increase the number of heart transplants [5, 6]. Studies on transplantation with ischemic time >6 hours are few and demonstrate the ambiguous influence of this expanded donation factor on immediate and long-term outcomes of HTx [7, 8].

The **objective** of the study was to determine the effect of extremely prolonged ischemic time (>6 hours) on the nature of restoration of primary function in heart transplant recipients and the immediate outcomes of HTx.

MATERIALS AND METHODS

During the period from January 1, 2011 to December 31, 2021, 1500 heart transplant surgeries were performed at Shumakov National Medical Research Center of Transplantology and Artificial Organs, Moscow. This included 38 (2.5%) with ischemic time >360 minutes. The study included 38 recipients (33 (86.8%) men and 5 (13.2%) women) aged 11 to 66 (44.7 ± 12.0, median 48.0 years), who underwent primary (n = 37 (97.4%)) or repeat (n = 1 (2.6%)) HTx (retransplantation) with a given ischemic time. In all observations, transplantation with extremely prolonged (≥6 hours) donor heart ischemic time was due to the territorial distance of the donor base from the transplant center.

Pre-transplant characteristics of the heart recipient

The main cardiac conditions that led to end-stage chronic heart failure (CHF) and the need for HTx were dilated cardiomyopathy (n = 20 (52.6%)), coronary heart disease (n = 16 (42.1%)), restrictive cardiomyopathy (n = 1 (2.6%)), and long-term irreversible heart graft dysfunction (n = 1 (2.6%)). The severity of CHF corresponded to classes IIA (n = 2 (5.3%)), IIB (n = 25 (65.8%)), and III (n = 11 (28.9%)) according to the Strazhesko–Vasilenko classification or 3 (n = 4 (10.5%)) and 4 (n = 34 (89.5%)) functional class (3.8 ± 0.4) according to the

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NYHA classification. The urgency of HTx corresponded to status IA (n = 18 (47.4%)), IB (n = 5 (13.2%)), or 2 (n = 15 (39.4%)) according to UNOS.

Eleven (28.9%) patients had heart arrhythmia in the form of permanent atrial fibrillation. A cardioverter defibrillator was implanted in 8 (21.1%) recipients. Five (13.2%) recipients had previously undergone cardiac surgery on the open chest and pericardial cavity: implantation of a long-term left ventricular bypass system (n = 3); coronary artery bypass grafting (n = 1); primary HTx (n = 1).

Concomitant conditions included: class 2 obesity (n = 8 (21.1%); arterial hypertension (n = 7 (18.4%); multifocal atherosclerosis with lesions of brachycephalic and/or lower extremity arteries (n = 7 (18.4%)); chronic bronchitis (n = 5 (13.2%)); gastric/duodenal ulcer (n = 5 (13.2%)); dyscirculatory encephalopathy (n = 4 (10.5%)); gout (n = 4 (10.5%)); subclinical hypothyroidism (n = 3 (7.9%)); cholelithiasis (n = 3 (7.9%)); urolithiasis (n = 3 (7.9%)); chronic kidney disease stage 2 and higher (n = 3 (7.9%)); condition after acute cerebrovascular disease (n = 2 (5.3%)); type 2 diabetes mellitus (n = 2 (5.3%)); condition after pulmonary embolism (n = 1 (2.6%)).

In 5 (13.2%) patients, dopamine (3–6 (3.9 ± 1.6) μg/kg/min (n = 4)) or dobutamine (4 μg/kg/min (n = 1)) cardiotonic therapy was sufficient to correct systemic hemodynamic disorders; which lasted for 4–30 (7.1 ± 10.1) days before HTx.

In 15 (39.5%)) recipients, we used short-term pre-transplant mechanical circulatory support (MSC) by peripheral venoarterial extracorporeal membrane oxygenation (VA-ECMO), in 4 (10.5%)) – prolonged MSC by implanted left ventricular bypass. VA-ECMO before HTx lasted for 1–6 (2.1 ± 0.8) days.

The clinical, laboratory, and instrumental pre-transplant examination of recipients, including the results of invasive central hemodynamic studies at the time of inclusion on the heart transplant waitlist, are presented in Table 1.

Clinical, instrumental, and laboratory examination of heart donors

Heart was harvested from brain-dead donors (n = 38), whose brain death was caused by nontraumatic (n = 30 (78.9%)) or traumatic (n = 8 (21.1%)) lesion. The harvesting was done at donor centers located in the following places: Voronezh (n = 10 (26.3%)); Tula (n = 5 (13.2%)); Arkhangelsk (n = 4 (10.5%)); Ryazan (n = 4 (10.5%)); Volgograd (n = 3 (7.9%)); Samara (n = 3 (7.9%)); Tyumen (n = 3 (7.9%)); Rostov-on-Don (n = 2 (5.3%)); Ivanovo (n = 1 (2.6%)); Kazan (n = 1 (2.6%)); Chelyabinsk (n = 1 (2.6%)); Ufa (n = 1 (2.6%)). In 28 (73.7%) and 10 (26.3%) observations, long-distance transportation of donor heart was done by air and road transport, respectively.

The age of heart donors (29 (76.3%) men and 9 (23.7%) women) was 22–60 (41.6 ± 9.7) years, including 4 (10.5%) donors aged 55 years or above; weight was 60–110 (78.4 ± 12.1) kg, and artificial ventilation (AV) lasted for 1–9 (2.2 ± 1.6) days. None of the donors had cardiopulmonary resuscitation episodes. The main parameters of clinical, laboratory and instrumental examination of the heart donors are presented in Table 2.

The donor heart was cold preserved with histidine-tryptophan-ketoglutarate (Custodiol®) solution by non-selective antegrade cardioplegia in a 3–4 L volume depending on the donor's anthropometric parameters. Repeated injection of 1 L of chilled the preservative solution was performed immediately before donor heart suturing through a cardioplegic cannula placed in the ascending aorta before the first injection of the preserving solution.

The criteria for expanded heart donation were (1) donor age >50 years; (2) left ventricular hypertrophy ≥1.4 cm; (3) left ventricular ejection fraction <50%; (4) high sympathomimetic vasopressor/cardiotonic support (norepinephrine >600 ng/kg/min or dopamine >10 μg/kg/min); (5) sustained cardiopulmonary resuscitation >5 min; (6) transient (lifetime) coronary artery atherosclerosis; (7) potentially correctable cardiac valve pathology; (8) hypernatremia >160 mmol/L; (9) methanol poisoning [9].

The following prognostic scales were used to objectively assess the degree of donor heart marginality and the risk of primary graft dysfunction: Eurotransplant Donor Heart Scale [10], Donor Risk Index Model [11], RADIAL score [12]. A heart donor was qualified as having expanded criteria if there were more than 17 points on the Eurotransplant Donor Heart Scale and 9 points or more on the Donor Risk Index Model. Incidence of primary heart graft dysfunction according to the RADIAL score was estimated according to the total score: 0 point, 2.1%; 1 point, 4.1%; 2 points, 8.1%; 3 points, 15.2%; 4 points, 27.4%; ≥5 points, 44.2% [12].

We quantified the magnitude of inotropic/vasopressor therapy using the Wernovsky-Inotropic Score (WIS) = dopamine (μg/kg/min) + dobutamine (μg/kg/min) + 100 × adrenaline (μg/kg/min) and Vasoactive Inotropic Score (VIS) = WIS + 10 × milrinone (μg/kg/min) + vasopressin (U/kg/min) + norepinephrine (μg/kg/min) [13, 14].

Early heart graft dysfunction was classified as primary or early secondary dysfunction. The diagnosis and severity of primary graft dysfunction was established in accordance with ISHLT criteria from 2010 [15]. Early secondary graft dysfunction was defined as impaired pumping function in heart transplant recipients that developed in the early post-transplant period and was due to immunological reasons, high pulmonary hypertension or errors in heart transplant surgical technique [16].

Table 1

Preoperative characteristics of heart recipients who underwent transplantation with donor heart ischemia time of more than 6 hours (n = 38)

Parameter	Value (minimum, maximum, mean)
Non-invasive and invasive (right heart catheterization) hemodynamic assessment at the time of inclusion in the waiting list	
HR, bpm	54–120 (79.3 ± 17.1)
Systolic BP, mm Hg.	86–144 (106.0 ± 14.1)
Diastolic BP, mm Hg.	48–92 (69.6 ± 11.8)
Mean BP, mm Hg.	60–101 (78.2 ± 11.7)
RAP, mm Hg.	4–19 (8.8 ± 4.5)
Systolic PAP, mm Hg.	29–51 (36.9 ± 12.1)
Diastolic PAP, mm Hg.	10–35 (20.4 ± 7.5)
Average PAP, mm Hg.	15–44 (25.1 ± 8.1)
PCWP, mm Hg.	11–32 (18.9 ± 6.6)
CO, L/min	2.2–5.1 (3.5 ± 0.9)
CI, L/min/m^2	1.82 ± 0.41 (1.2–2.3)
TPG, mm Hg.	2–12 (7.0 ± 2.8)
PVR, Wood units	0.6–5.9 (2.3 ± 1.2)
Laboratory tests within 24 hours before heart transplantation	
Hemoglobin, g/dL	11.0–16.7 (13.3 ± 2.5)
White blood cells, 10^9/L	5.7–13.6 (7.8 ± 2.7)
Platelets, 10^9/L	74–396 (173.2 ± 70.5)
Urea, mmol/L	6.0–16.7 (8.1 ± 3.3)
Creatinine, μmol/L	48–152 (100.7 ± 31.2)
Total bilirubin, μmol/L	11–95 (33.1 ± 23.3)
ALT, IU/L	22–175 (30.7 ± 31.8)
AST, IU/L	16–146 (35.7 ± 32.9)
Total protein, g/L	59–87 (72.2 ± 6.5)
Glucose, mmol/L	4.4–8.6 (5.9 ± 1.2)
PI, %	48–97 (83.3 ± 10.2)
INR	1.1–2.4 (1.38 ± 0.40)
K^+, mmol/L	3.2–4.9 (3.6 ± 0.4)
Na^+, mmol/L	126–140 (134.5 ± 3.5)
pH	7.30–7.49 (7.40 ± 0.08)
BEa, mmol/L	(–) 3.5–3.6 (0.59 ± 3.0)
Lactate, mmol/L	0.6–1.7 (1.1 ± 0.4)
Transthoracic echocardiography within one week before heart transplantation	
Ascending aorta, cm	1.8–3.5 (3.1 ± 0.6)
Left atrium, cm	4.2–6.6 (5.1 ± 0.7)
Right ventricle, cm	1.8–4.4 (3.2 ± 0.7)
LVEDD, cm	4.5–7.5 (6.3 ± 1.2)
LVESD, cm	2.9–6.8 (5.1 ± 1.5)
LVESV, mL	88–360 (231.1 ± 82.4)
LVEDV, mL	48–221 (177.9 ± 77.1)
SV, mL	18–71 (54.4 ± 23.1)
LVEF	10–33 (26.2 ± 14.1)
LVPW, cm	0.95 ± 0.17
IVS, cm	0.96 ± 0.17
Mitral regurgitation, grade	1.5–3.0 (1.9 ± 0.8)
Tricuspid regurgitation, grade	1.0–3.0 (2.1 ± 0.6)

Note: HR, heart rate; BP, blood pressure; RAP, right atrial pressure; PAP, pulmonary artery pressure; PCWP, pulmonary capillary wedge pressure; CO, cardiac output; CI, cardiac index; TPG, transpulmonary pressure gradient; PVR, pulmonary vascular resistance; ALT, alanine aminotransferase; AST, aspartate aminotransferase; PI, prothrombin index; INR, international normalized ratio; BEa, base excess arterial; LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter; LVESV, left ventricular end-systolic volume; LVEDV, left ventricular end-diastolic volume; SV, stroke volume; LVEF, left ventricular ejection fraction; LVPW, left ventricular posterior; IVS, interventricular septum.

The diagnosis and severity of donor-transmitted atherosclerosis was established according to the results of the first post-transplant coronary angiography performed no later than 1 month after HTx. The diagnosis and severity of acute cellular and antibody-mediated rejection were established according to ISHLT criteria [17, 18].

Statistical processing of the study data was performed using Microsoft Excel spreadsheets and Statistica for Windows 7.0 application software package (Start Soft Inc. USA), Biostat and SPSS. Normality of distributions was assessed using the Kolmogorov–Smirnov test. Mean values of numerical parameters were presented as $M \pm \sigma$. Mean values were compared using the Mann–Whithey U-test or Student's t-test. A significant difference was considered at $p < 0.05$. Pearson's chi-squared test and Fisher's exact test were used to compare frequencies of binary outcomes. The Kaplan–Meier estimate was used to assess survival, and survival was compared using the log-rank test.

RESULTS

The ischemic time of donor hearts (n = 38) ranged from 362 (6 hours 2 minutes) to 571 (9 hours 31 minutes) or 407 ± 52 (median 400) minutes, including: 361–420 minutes (7 hours), n = 27 (71.1%); 421–480 minutes (8 hours), n = 7 (18.4%); 481–540 minutes (9 hours), n = 3 (7.9%); >540 minutes (or >9 hours), n = 1 (2.6%).

The number of expanded heart donor factors was 2.2 ± 1.2. The degree of cardiac donor marginality according to the Eurotransplant Donor Heart Score was 19.2 ± 8.2, the Donor Risk Index Model score was 6.7 ± 2.1, and the RADIAL scale score was 2.9 ± 1.0. The predicted primary graft failure rate, calculated using the RADIAL scale, was 16.4 ± 10.6%.

Table 2

Donor characteristics with ischemic time >6 hours (n = 38)

Parameter	Value (minimum, maximum, mean)
Sympathomimetic cardiotonic/vasopressor support	
No sympathomimetic support, n (%)	7 (18.4%)
Norepinephrine only, n (%)	24 (63.2%)
Norepinephrine + dopamine, n (%)	7 (18.4%)
Dopamine (max), μg/kg/min, (n = 7)	3–18 (10.1 ± 6.9)
Dopamine (before withdrawal), μg/kg/min, (n = 4)	2–15 (4.1 ± 2.8)
Noradrenaline (max), ng/kg/min, (n = 24)	50–1000 (430.0 ± 185.3)
Norepinephrine (before withdrawal), μg/kg/min, (n = 24)	100–800 (288.2 ± 146.5)
Laboratory tests	
Hemoglobin, g/dL	11.0–16.7 (13.3 ± 2.5)
Total protein, g/L	59–87 (72.2 ± 6.5)
Glucose, mmol/L	4.4–8.6 (5.9 ± 1.2)
K^+, mmol/L	2.7–5.9 (3.8 ± 0.6)
Na^+, mmol/L	126–140 (134.5 ± 3.5)
pH	7.29–7.56 (7.41 ± 0.25)
BEa, mmol/L	(–) 3.3–2.5 (0.9 ± 1.3)
Blood lactate, mmol/L	0.8–6.9 (1.8 ± 0.7)
Transthoracic echocardiography	
Ascending aorta, cm	2.4–4.7 (3.2 ± 0.7)
Left atrium, cm	2.4–5.7 (3.7 ± 0.8)
Right ventricle, cm	2.1–3.5 (2.6 ± 0.5)
LVEDV, mL	56–130 (105.5 ± 27.2)
LVESV, mL	20–58 (39.9 ± 13.5)
SV, mL	35–105 (63.9 ± 21.7)
LVEF	50–70 (61.9 ± 6.4)
LVPW, cm	0.9–1.4 (1.2 ± 0.2)
IVS, cm	0.9–1.5 (1.2 ± 0.2)
IVS ≥1.4 cm, n (%)	6 (15.8%)
Mitral regurgitation, degree	0.0–1.5 (1.1 ± 0.3)
Tricuspid regurgitation, degree	1.0–1.5 (1.2 ± 0.2)
sPAP (estimated), mm Hg.	19–42 (26.7 ± 0.7)

Note: BEa, base excess arterial; LVEDV, left ventricular end-diastolic volume; LVESV, left ventricular end-systolic volume; SV, stroke volume; LVEF, left ventricular ejection fraction; LVPW, left ventricular posterior; IVS, interventricular septum; sPAP, systolic pulmonary artery pressure.

In 31 (81.6%) of 38 recipients, HTx was performed by bicaval technique, in 7 (18.2%) – by biatrial technique. Anesthesia lasted for 6.6 ± 0.9 hours, surgical intervention lasted for 4.9 ± 0.5 hours, cardiopulmonary bypass (CPB) was 63–164 (148 ± 25) minutes, heart graft suturing lasted for 43 ± 8 minutes, the "removal of aortic unclamping – weaning from CPB" interval was 48 ± 20 minutes.

In 33 (86.8%) of 38 recipients, the early post-transplant period was characterized by satisfactory initial graft function. In this cohort of recipients, the highest adrenaline dose during follow-up was 58.7 ± 21.3 ng/kg/min, dopamine was 8.1 ± 2.5 μg/kg/min, and dobutamine was 7.0 ± 2.3 μg/kg/min, dopamine or dobutamine dose at the time of transfer from the intensive care unit (ICU) was 3.9 ± 0.3; the highest VIS score was 16.2 ± 3.9, the lowest and highest CI values were 2.6 ± 0.2 and 3.1 ± 0.3 L/min/m^2, respectively, the highest RAP/CVP value is 13.9 ± 2.1 mm Hg, average PAP 25.4 ± 6.3 mm Hg, PCWP was 16.3 ± 1.9 mm Hg, and the lowest and highest LVEF values were 52.3 ± 6.7% and 62.0 ± 4.6%, respectively. Levosimendan as an additional component of sympathomimetic cardiotonic therapy was used in 100% of cases. In 16 (42.1%) recipients, we used double sequential administration of the drug. Postoperative adrenaline administration lasted for 62.5 ± 18.7 hours; the interval to achieve a dosage of less than 5 μg/kg/min with dopamine or dobutamine monotherapy was 4.9 ± 0.8 days; postoperative AV lasted for 12.5 ± 6.7 hours; treatment in the ICU was 5.7 ± 4.4 days; cardiotonic therapy in the early posttransplant period was 9.2 ± 5.2 days.

In 10 (26.3%) patients, a sinus rhythm was registered since the initial heart graft function was restored. Due to bradyarrhythmia in the heart transplant recipients, 28 (73.7%) the patients required temporary pacing in VOO mode (n = 6), AOO mode (n = 13) or VOO with transition to AOO mode (n = 9) with a generated HR of 100 to 120 per minute.

Five (13.1%) recipients developed severe primary graft dysfunction, necessitating post-transplant VA-ECMO (n = 4 (10.5%)) or prolongation of pre-transplant VA-ECMO for 8 days after HTx (n = 1 (2.6%)) (severe according to the ISHLT Primary Graft Dysfunction classification (2010)). Four (80.0%) of 5 recipients were diagnosed with a biventricular primary heart transplant dysfunction, and 1 (20.0%) had a predominantly right ventricular variant in the absence of pre-transplant pulmonary hypertension. Persistent resolution of severe primary graft dysfunction in 4 (80%) of 5 allowed termination of VA-ECMO at days 4–8 (6.1 ± 1.6) from the start of MSC.

According to the first coronary angiographic study, donor-transmitted atherosclerosis requiring percutaneous coronary intervention was detected in 3 (7.9%) of 38 recipients and included hemodynamically significant (over 50%) narrowing of 1 (n = 2) and 3 (n = 1) coronary arteries.

According to results from the first endomyocardial biopsy, acute cellular rejection grade 2 R or higher and/or antibody-mediated rejection pAMR grade 2 or higher was not diagnosed in any of the cases.

Hospital mortality was 7.9% (n = 3). In all cases, the cause of death was progressive multiple-organ failure developed against the background of severe primary dysfunction of the heart transplant (n = 1) and purulent-septic complications (bacterial pneumonia (n = 1), pancreatic necrosis (n = 1)). Hospital mortality in the cohort of recipients with severe primary dysfunction was 20% (1 in 5).

Thirty-five (92.1%) of 38 recipients were discharged from the hospital. The duration of ICU treatment among the surviving recipients was 5.8 ± 1.4 days. The follow-up period at the end of data collection (December 31, 2021) was 1053 ± 174 days. Three recipients died in the posthospital period at days 734, 944, and 2146 after HTx. The causes of death were lung cancer (n = 1), sepsis and multiple-organ failure against the background of pneumonia developed in out-of-hospital conditions (n = 1), and sudden death (n = 1). Of the 38 recipients, 32 (84.2%) were still alive at the end of the study. The mean life expectancy of recipients with prolonged ischemic time was 70.7 ± 5.6 months at the end of the study (Fig.).

DISCUSSION

Prolonged preservation of donor heart may be due to the time it takes to transport the donor heart from the donor base to the transplant center, or due to a delay in suturing the donor heart as a result of prolonged isolation (cardiolysis) of the recipient's own heart in the repeated nature of surgical intervention (for example, explantation together with removal of the implanted assisted circulation system) or other reasons, leading to prolonged time interval between removal and beginning of suturing of the donor heart [19].

Suspected prolonged ischemic time is one of the "traditional" criteria for expanded heart donation [20]. Amid the current donor organ shortage over the last three decades, the use of hearts from expanded criteria donors, including those with prolonged ischemic time, remains a feasible way to increase the availability of heart transplantation, including in patients who need it urgently and/or have a predicted worse early and long-term post-transplant survival, independent of donor characteristics [21].

The limits of acceptable duration of donor heart ischemic time have not yet been defined and are the subject of scientific research. The threshold cold preservation time for the donor heart is considered to be 4 hours [22, 23]. According to the guidelines of the International Society for Heart and Lung Transplantation (ISHLT), transplantation with a donor ischemic time >4 hours is allowed

in certain clinical situations when other heart donor factors are ideal for effective HTx (young age, normal systolic function, no inotropic support) [22]. Earlier studies have shown that donor ischemic time >4 hours significantly increases the risk of severe primary graft dysfunction requiring the use of mechanical circulatory support (MCS) [24]. Some transplant centers consider it acceptable to perform HTx with donor ischemic time of 4–6 hours [20, 25]. Cases of HTx with donor ischemic time >6 hours are rare and, as a rule, are done at transplantation centers with experience in performing these transplants and/or heart transplants from expanded criteria donors [5, 26, 27].

It is well known that cold cardioplegia, which is the main method of donor heart preservation, does not provide complete cessation of metabolic processes in myocardium in conditions of its anoxia, leading to depletion of energy substrates, intracellular acidosis, hyperproduction of reactive oxygen species and cardiomyocyte edema [28]. Subsequent reperfusion (re-oxygenation) enhances the functional and morphological damage to the heart transplant myocardium. The leading pathogenetic mechanisms of ischemia-reperfusion injury (IRI) in heart transplant recipients are hyperproduction of reactive oxygen species and calcium overload, which leads to uncontrolled activation of calcium-dependent ion transport systems, depletion of energy reserves, disruption of cardiomyocyte metabolism and subsequent irreversible cardiomyocyte damage [29]. Disruption of mitochondrial calcium-dependent pores or mitochondrial permeability transition pores plays an important role in the chain of pathophysiological disorders caused by IRI [30, 31]. As it increases, the potentially negative influence of ischemic time on functional and morphological disorders of heart graft caused by IRI, as well as on immediate and long-term outcomes of HTx, increases [32].

Prolonged preservation increases the risk of severe primary dysfunction in heart transplant recipients. The leading cause is a combination of irreversible and reversible ischemic-reperfusion myocardial injury to the cardiac graft [33]. Primary graft dysfunction remains the most common cause of death in the early stages after HTx [34]. The risk of severe primary dysfunction increases when prolonged ischemic time is combined with other expanded heart donation factors (e.g., age of the donor) [35]. Hearts from young donors (age <34 years) are more tolerant to prolonged ischemic time compared to hearts from older donors (>34 years), which predetermines better early and long-term survival after HTx [36]. The relationship between ischemic time and the risk of acute graft rejection, as well as accelerated coronary artery disease in heart transplant recipients and chronic dysfunction in the long term after HTx has been revealed [37].

According to the multicenter, international ISHLT registry (2017), 18,772 HTx were performed between January 2009 and June 2015, of which 1.8% (n = 337) were with ischemic time >6 hours [32]. In the study we presented, the proportion of transplants with donor ischemic time >6 hours was 2.5% between January 1, 2011 and December 31, 2021. Almost half of the patients (47.4%) required urgent HTx, including 39.5% (n = 15) with short-term pre-transplant MCS and 5.3% (n = 2) with life-threatening complications of long-term MCS (implantable left ventricular bypass systems).

Severe early graft dysfunction requiring MCS developed in 13.1% of observations, corresponding to its predicted incidence according to the RADIAL scale

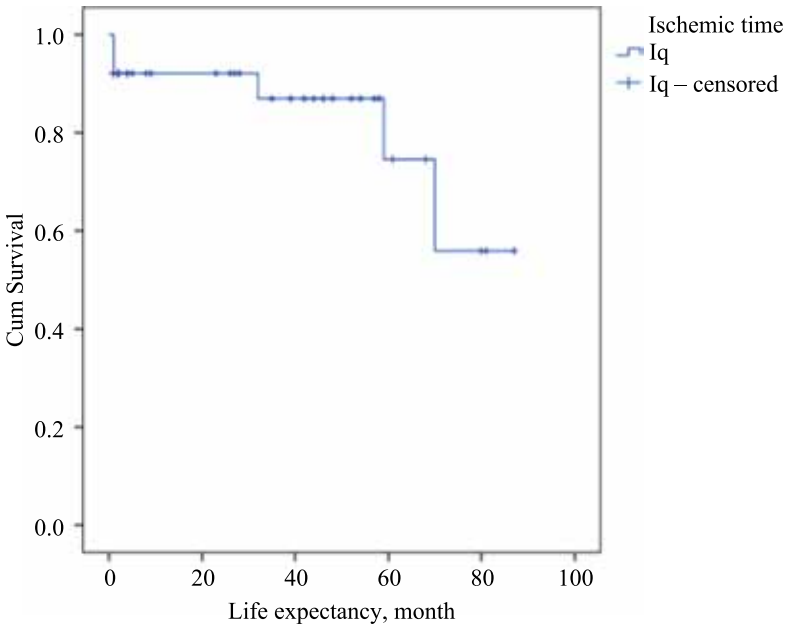

Fig. Kaplan–Meier estimates of survival in heart recipients with graft ischemic time >6 hours

(16.4 ± 4.6%). A meta-analysis by Buchan T.A. et al (2021) found that the incidence of primary graft dysfunction in heart transplant recipients was 20.5% according to ISHLT classification (2010), of which 7.7% were for severe dysfunctions requiring MCS [34]. Increasing the donor ischemic time beyond 240 minutes increases by three times the risk of primary graft dysfunction [38]. Starting from the threshold value of 240 minutes, further increase in ischemic times leads to a linear increase in the incidence of primary graft dysfunction in heart transplant recipients [34]. In an earlier study by Marasco S.F. et al (2007), increasing ischemic time from 240 minutes to 360 minutes or more increases the incidence of early graft dysfunction by 2.9 times (from 17% to 50%), the frequency of post-transplant MCS by 4.4 times (from 7% to 31%) and the median ICU treatment duration by 3.3 times (from 3 days to 10 days) [39]. Thus, according to Marasco S.F. et al (2007), every second recipient develops primary dysfunction when the ischemic time increases over 6 hours, and every 3 recipients need MCS at these graft ischemic times [39]. Buchan T.A. et al (2021) found that increasing the ischemic time by 1 hour increases the incidence of primary dysfunction by 1%, and increasing the age of the heart donor by 10 years, increases the incidence by 65% [34]. Pre-transplantation use of VA-ECMO is associated with a 10-fold increase in the incidence of primary graft dysfunction [40].

In-hospital mortality rates in recipients with primary dysfunction vary widely (from 19% to 37%) and in most studies depend on the severity of its hemodynamic manifestations [41]. In our study, the in-hospital mortality of recipients with primary graft dysfunction requiring MCS was 20% (1 of 5) or 33.3% of all cases (1 of 3) of in-hospital mortality in transplantation with donor ischemic time >6 hours.

In-hospital patient survival in extremely (>6 hours) prolonged ischemic time was 92.1%, which is comparable to those (93%) in HTx within the recommended ischemic time (<240 minutes) [39].

CONCLUSION

1. 2.53% of heart transplants were performed with an ischemic time >6 hours, which in all cases was due to the territorial distance of the donor base from the transplant center.
2. In transplantations with excessively prolonged (>6 hours) graft ischemic time, the incidence of severe early dysfunction in the heart transplant recipients, which required MCS (venoarterial extracorporeal membrane oxygenation) was 13.1%.
3. In-hospital survival of transplant recipients with excessively prolonged (>6 hours) ischemic time was 92.1%.

The authors declare no conflict of interest.

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HEART TRANSPLANTATION FOR PRIMARY CARDIAC SARCOMA

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Primary cardiac sarcoma is a rapidly progressive, aggressive cardiac tumor that is very rare in the general population. Conservative treatment for this tumor is not very effective. The only effective treatment is radical surgical removal of the malignancy. However, if sarcoma removal cannot be performed, heart transplantation (HT) becomes the only treatment option. The median survival of heart recipients with preoperative cardiac sarcoma is 8.5 months. Globally, such operations are performed in a small number. This paper presents the first experience of HT for a patient with primary cardiac sarcoma in the Russian Federation.

Keywords: heart transplantation, primary cardiac sarcoma, immunosuppressive therapy.

INTRODUCTION

Primary cardiac tumors are a rare entity with an overall incidence of 0.001–0.02% of all cardiac tumors [1, 2]. According to reports, the disease has an unfavorable prognosis, and the only effective method of treatment remains radical surgical removal of the tumor, if it is possible [3]. If it is an unresectable cardiac sarcoma (CS), HT becomes the only radical method of treatment [4]. Currently, there are data on a small number of cases of patients with CS with no regional and distant metastases, who have undergone HT. The role of HT in patients with CS is controversial. For example, Coelho P. et al. cite data from the clinical case of a patient with CS who underwent HT. The authors draw attention to the fact that the survival rate of patients with CS without surgical treatment is 9–11 months. The follow-up time of the heart recipient in this study was 7 years. The authors showed that HT can be a successful method of treatment for unresectable and non-metastatic CS [6]. On the contrary, Jimenez Mazuecos J.M. et al. in their study including 8 CS patients could not find any advantages of HT in this category of patients. The authors found no significant differences in survival between the groups of CS patients who did not undergo HT and those who underwent HT (11 and 12 months, respectively) [7]. In the Russian Federation, no cases of HT for CS have been registered so far, so this clinical case is important.

CLINICAL CASE STUDY

Recipient baseline data

Patient U., a 17-year-old male, grew and developed according to his age. In September 2019, a mass was detected in the cardiac apex region by a chest X-ray for the first time. The child was consulted by a tuberculosis specialist and no evidence of tuberculosis was obtained. During a medical examination in September 2020, ECG revealed repolarization changes in the form of negative T waves in leads III, aVF, and V3–V6; no further follow-up examination was performed, no medications were prescribed. He was infected by COVID-19 in October 2020, and was examined by a cardiologist in December 2020, taking into account the above-described changes on ECG; he was diagnosed with acute myocarditis. The patient was admitted to a hospital located at his place of residence. Echo findings: cardiac chambers were not enlarged, contractility was preserved, left ventricular (LV) hypertrophy was not detected, a volume echo-positive mass measuring 76 × 48 × 39 mm was detected in the apex region. Heart MRI was performed as a follow-up examination, which revealed a heterogeneous neoplasm, emanating from the LV myocardium at the diaphragm dome level, with clear and even contours, without perifocal infiltration, measuring 89 × 65 × 65 mm, caudally displacing 1.7 cm and compressing the diaphragm; regional lymph nodes not enlarged (Fig. 1).

In January 2021, he underwent a scheduled in-patient examination. Echo findings: a crescent-shaped mass surrounded the inferolateral and posterolateral surface of the LV and the posterior wall of the right ventricle (RV), heterogeneous, with areas of dissection. Compared to the study in December 2020, the size of the tumor slightly increased and was 95 × 65 × 67 mm with no apparent vascularization.

In March 2021, the patient was admitted to the Center for the Treatment of Women, Children and Youth with a perinatal center and the Charité center for Genetics (Berlin, Germany). On March 18, 2021, percutaneous

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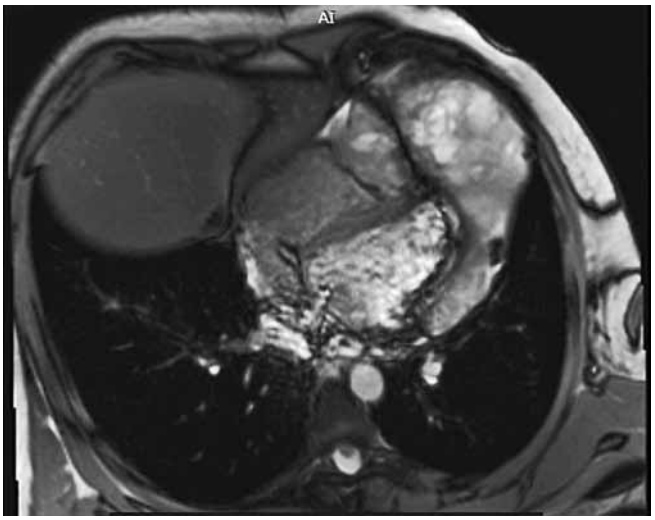

Fig. 1. Cardiac tumor detected on a heart MRI in patient U., 17 years old

CT-guided needle biopsy of the heart tumor was performed. Biopsy findings: malignant cardiac spindle cell tumor, with no clear linear differentiation, about 340 mL in volume, myocardial infiltration through spindle cell neoplasia. A study for the presence of tumor metastases in organs and systems was performed, no evidence of metastases was found.

Antineoplastic polychemotherapy (PCT) was chosen as the conservative therapy. The PCT regimens and duration are shown in Table 1.

Given that radical surgical treatment was not possible, the patient was consulted in absentia at the Shumakov National Medical Research Center of Transplantology and Artificial Organs (Shumakov Research Center). In August 2021, he was examined at Shumakov Research Center. Based on the results of clinical and laboratory examination, the patient was placed on the heart transplant waiting list and was discharged in a stable condition.

After discharge at his place of residence, second-line chemotherapy (VIT blocks) was continued. Due to development of leukopenia (2.14 × 10^9/L), granulocytopoiesis stimulation with filgrastim was performed.

Table 1

Antineoplastic polychemotherapy regimens and duration

S/N	PCT block regimen	PCT block composition	Duration
1	I2VA (5 courses)	Vincristine Actinomycin Ifosfamide	31/03/2021–16/06/2021 13/07/2021–14/07/2021
2	VIT	Vincristine Irinotecan Temodal	24/08/2021–16/09/2021 07/10/2021–11/10/2021 29/10/2021–02/11/2021

Heart transplantation and early postoperative period

On November 27, 2021, the patient (initial weight and height – 66 kg and 182 cm) underwent bicaval orthotopic heart transplantation (OHT). The donor was a 48-year-old woman (height 170 cm, weight 80 kg). The cause of cerebral death was acute hemorrhagic stroke with ventricular rupture. Graft ischemic time was 317 minutes, cardiopulmonary bypass lasted for 171 minutes. HT proceeded typically. A peculiarity of the operation was that due to a pronounced adhesion process between the neoplasm and the left pericardial area, the tumor-like neoplasm was removed in a single block with the left pericardium and the recipient's heart (Fig. 2). Tracheal extubation was performed on postoperative day 1. After HT, temporary pacing and inotropic support with

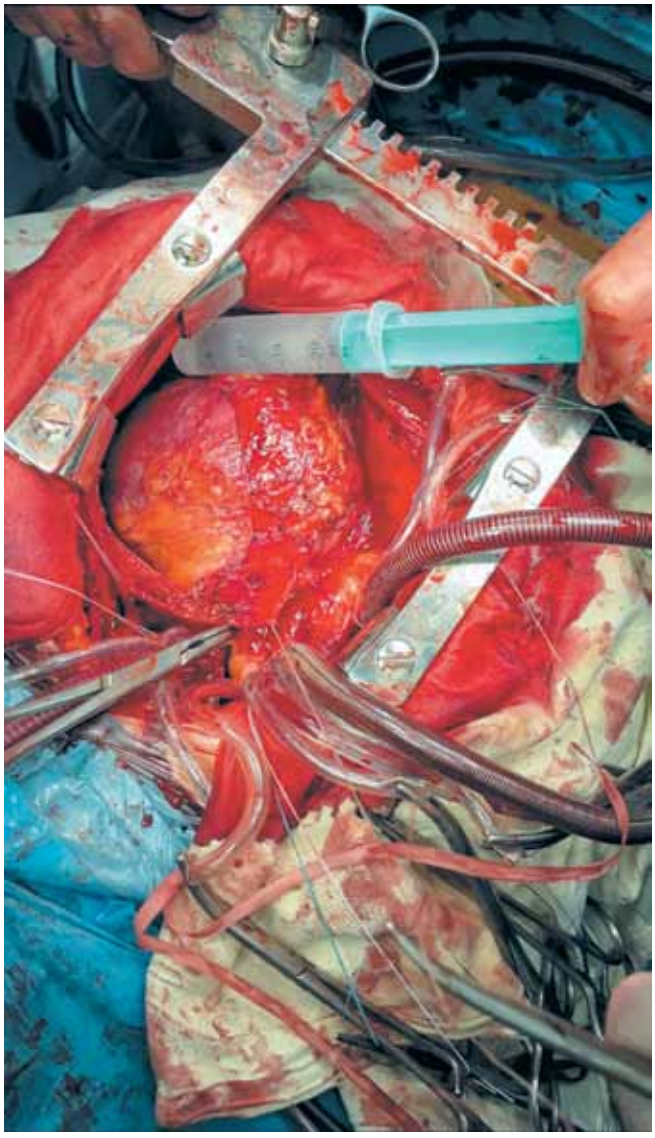

Fig. 2. Heart transplantation in patient U., 17 years old

dopamine at a 3 μg/kg/min dose with a gradual dose reduction against the background of resolving myocardial insufficiency were required.

Basiliximab was administered as induction according to the accepted guidelines for the management of heart transplant recipients.

Table 2

Postoperative period and survival in patients with malignant heart tumors after heart transplantation. Gowdamarajan et al.

Study	Age, Sex	PCT	Death	Follow-up (months)
Jamieson et al.	17 y.o., F	No	Yes	75
Horn et al.	13 y.o., M	Yes (before and after OHT)	Yes (metastases)	15
Aravot et al.	43 y.o., F	No	No	66
Aufiero et al.	31 y.o., F	No	No	12
Yuh et al.	57 y.o., F	Yes (after OHT)	Yes (metastases)	14
Baay et al.	34 y.o., M	Yes (before and after OHT)	No	33
Bachet et al.	35 y.o., M	No	Yes (relapse)	18
Demkow et al.	4 months, M	No	No	8
Mark et al.	2 y.o., F	No	Yes (rejection)	8
Crespo et al.	31 y.o., M	Yes (before OHT)	Yes (metastases)	8
	32 y.o., M	Yes (before and after OHT)	Yes (metastases)	9
Valente et al.	38 y.o., F	No	No	36
	40 y.o., F	No	No	28
Siebermann et al.	31 y.o., F	No	Yes (metastases)	2
Michler	42 y.o., F	Yes (before and after OHT)	No	6
	49 y.o., F	Yes (before OHT)	No	34
	26 y.o., F	No	No	60
	49 y.o., F	No	No	38
	39 y.o., F	Yes	Yes	3.5
	3.5 months, F	No	No	105
Almenar	29 y.o., F	Yes (before OHT)	Yes	2
Noirclerk	unknown	Yes (before OHT)	No	20
Gowdamarajan et al.	64 y.o., M	No	Yes	3
	7.5 y.o., M	Yes	Yes	11.5
	28 y.o., F	No	Yes	11.5
	9 y.o., M	No	Yes	11.5
	61 y.o., F	No	Yes	36
	8 y.o., M	Yes (after OHT)	Yes	21

Note: M, male; F, female; OHT, orthotopic heart transplantation; y.o., years old; PCT, palliative chemotherapy.

Table 3

Postoperative and survival in patients with malignant heart tumors after heart transplantation. Li H. et al.

Age / Sex	Histology (Grade)	Tumor location	Pre-OHT surgeries / Interval (months)	PCT (before and after OHT)	Tumor relapse (months)	Survival (months)
63 / M	Synovial sarcoma (G3)	LV, RV	Partial resection / 7	Yes (before and after OHT)	Lungs (1)	Death (5)
48 / M	Angiosarcoma (G3)	RA, RV	Biopsy / 3	Yes (after OHT)	Liver, Chest (4)	Death (5)
27 / F	Angiosarcoma (G3)	RA, RV	Biopsy / 2	Yes	Lungs (12)	Death (15)
49 / F	Undifferentiated pleomorphic sarcoma (G3)	RA, LA, LV	Partial resection / 5	Yes	PV (33); Liver, PV (40)	Death (43)
49 / F	Undifferentiated pleomorphic sarcoma (G2)	LA, LV	Partial resection / 9	No	No	Death from acute rejection / 18
61 / M	Myxoid liposarcoma (G2)	RV	Partial resection / 3	No	No	Alive / 93

Note: M, male; F, female; OHT, orthotopic heart transplantation; LV, left ventricle; RV, right ventricle; LA, left atrium; RA, right atrium; PV, pulmonary vein; PCT, palliative chemotherapy.

The patient was transferred to the ward on postoperative day 3 for follow-up and treatment. According to echocardiography at the time of transfer to the ward, global systolic function of the left ventricle was satisfactory (LV ejection fraction 62%). Against the background of resolving right ventricular insufficiency, the dose of inotropic support with dopamine was gradually decreased. In this case, a triple immunosuppressive therapy was used, which included a combination of calcineurin inhibitors (tacrolimus), antimetabolites (mycophenolate mofetil), and corticosteroids (methylprednisolone).

Coronary angiography and endomyocardial biopsy were performed to exclude graft rejection and transmissible atherosclerosis. Endomyocardial biopsy detected no acute cellular and antibody-mediated graft rejection; no stenotic lesion of the graft coronary arteries was diagnosed by coronary angiography.

After discharge from the Shumakov Research Center, ribociclib therapy was initiated at a dose of 600 mg once a day in a cycle of 21–28 days, with six cycles planned in total.

Given the patient's history of CS, immunosuppressive therapy was converted to the "tacrolimus-everolimus-methylprednisolone" regimen, followed by conversion to "tacrolimus-everolimus" immunosuppressive therapy.

Three months after transplantation, the immunosuppressive therapy protocol was changed to the "tacrolimus-everolimus-methylprednisolone" regimen under control of tacrolimus and everolimus blood concentrations, the target values were achieved (tacrolimus concentration 5.31 ng/mL, everolimus concentration 3.24 ng/mL). According to a control endomyocardial biopsy dated March 23, 2022, no acute graft rejection was detected. Echo showed no evidence of graft dysfunction.

DISCUSSION

CS is one of the most rarely detected malignant heart tumors with the most aggressive course and unfavorable prognosis in patients. HT is the only radical method of treatment in patients with unresectable and non-metastatic CS. HT on one hand allows to increase the length and quality of life of a patient, but the use of immunosuppressive therapy is a risk factor for early tumor recurrence [8]. Also, the need for chemotherapy in the postoperative period [9] has a negative impact on the heart graft. Life expectancy of heart transplant recipients in this category of patients averages from 9 to 36 months [10]. It should also be taken into account that there are no special therapy regimens and clinical guidelines for the treatment of patients after HT for CS; managing such patients is on an individual basis, taking into account all the features of the postoperative period [11].

In world practice, there are a small number of cases of patients with non-metastatic CS, who underwent HT. For example, Gowdamarajan A. et al. in 2000 published a literature review, which included 28 patients aged from 4 months to 64 years [4]. Results of the studies are shown in Table 2.

In 2016, Li H. et al. published their study, which included 46 CS patients (40 patients were included in the study based on literature sources, 6 patients were the clinic's own observation) who underwent HT, as well as 7 CS patients who received palliative therapy [5]. Table 3 presents data from clinical cases (6 heart recipients) by Li H. et al.

The one-year, two-year, and five-year survival rates of the heart recipients (n = 46) were 61% ± 7%, 44% ± 8%, and 26% ± 8%, respectively. There was no significant difference (p = 0.768) between the median survival of the 6 recipients in Table 3, which was 15 months (5 to 93 months) and that of the remaining 40 recipients, which was 16 months (2 to 112 months). This study showed that neoadjuvant or adjuvant chemotherapy did not confer a survival advantage after HT.

The immunosuppressive therapy regimen in the patients in the reports published so far was a triple therapy, including calcineurin inhibitors (cyclosporine, tacrolimus), azathioprine/mycophenolate mofetil and methylprednisolone.

The survival of CS patients who underwent HT in all of these studies is significantly lower than the survival of recipients after HT without a history of CS; this makes it necessary to perform HT in CS patients [12]. Foreign reports show that marginal donors could be used as a more acceptable strategy for managing such patients [5].

However, in most foreign studies, authors point to the need for HT in patients with CS depending on the patient's clinical status. In the absence of large, randomized trials, it is important to determine the necessity and effectiveness of HT in each individual case. There is also no consensus on the need for chemotherapy courses after HT. This has forced researchers to decide empirically on the treatment and management of patients after HT.

By the time of writing this paper, the follow-up period in our case was 10.5 months. Further observation and publication of this clinical case will allow us to draw conclusions about the effectiveness of the therapy, graft function, and long-term outcomes in this patient.

The authors declare no conflict of interest.

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RESULTS OF PRECLINICAL TRIALS IN A SHEEP MODEL OF BIODEGRADABLE SMALL-DIAMETER VASCULAR GRAFTS

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Surface modification of polymer vascular matrices is a promising development for preventing vascular graft thrombosis, improving long-term patency and accelerating remodeling. **Objective:** to study the outcomes of long-term patency of PHBV/PCL/GFmix grafts with iloprost (Ilo) and heparin (Hep) implanted into the carotid artery of sheep. **Materials and methods.** Matrices ∅4 mm were fabricated by electrospinning from a polymer composition of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) and poly(ε-caprolactone) (PCL) with incorporation of endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF) and chemoattractant molecule (SDF-1α). The fabricated matrices were then modified with Ilo and Hep by complexation via polyvinylpyrrolidone (PVP). Synthetic Gore-Tex grafts were used as a comparison group. The physical and mechanical properties of the studied matrix groups were evaluated, the surface structure of vascular grafts before and after implantation was assessed. Vascular grafts were implanted into the carotid artery of a sheep. The explanted samples were studied via histological and immunofluorescence analysis, the elemental composition of the obtained vascular graft samples was also assessed, and the gene expression profile was evaluated. **Results.** One day after implantation, the patency of PHBV/PCL/GFmix$^{Hep/Ilo}$ vascular grafts was 62.5%, whereas synthetic Gore-Tex grafts had thrombosis in 100% of cases. At the same time, after 18 months of implantation, the patency of biodegradable PHBV/PCL/GFmix$^{Hep/Ilo}$ vascular grafts decreased to 50%. Permeable drug-coated polymer grafts were completely reabsorbed after 18 months of implantation, and aneurysmally dilated newly-formed vascular tissue was formed in their place. **Conclusion**. Modification of the surface of PHBV/PCL/GFmix polymer grafts with Hep + Ilo coating improved long-term patency outcomes compared to synthetic Gore-Tex grafts.

Keywords: vascular grafts, anti-thrombogenic coating, electrospinning, implantation, heparin, iloprost.

INTRODUCTION

At present, there has been a continuous increase in the incidence of atherosclerosis among the population, including coronary artery lesions and peripheral artery disease [1–5].

Therefore, there is an increasing number of surgical interventions using prosthetic implants, shunts and patches to restore effective blood flow in damaged blood vessels [6]. The best option for surgical treatment of the above pathology is the use of autologous material (own blood vessels), which is currently the gold standard. However, these grafts have limited availability due to previous operations with these vessels, progressive atherosclerosis and other diseases [7]. In addition, the graft harvesting process and subsequent evaluation before implantation can damage the vessel and lead to endothelial dysfunction, proinflammatory response and, ultimately, graft thrombosis and occlusion. High failure rates make this treatment option largely inadequate, leading to the development of non-autologous alternatives [8–10]. Xenogeneic and synthetic products currently used in clinical practice are well suited for large-diameter vascular grafts but are at high risk of thrombosis. For small-diameter vascular grafts, there is the risk of neointimal hyperplasia in the late postoperative period [7, 11–13].

In this regard, the lack of cardiovascular surgery products that are based on alternative materials and do not cause such complications is the most pressing problem. One of the promising modern fields involved in the development of vascular grafts is vascular tissue engineering [14–17], which allows using non-standard types of materials (biodegradable polymers, natural polymers, autologous biological fluids and tissues) for creating medical devices, as well as original approaches to their manufacturing, ensuring porosity of created structures and, consequently, effective migration of own cells into the wall of these structures to form in situ new healthy tissue [18–22].

However, due to high porosity and prolonged resorption involving monocyte-macrophage system cells, nonwoven biodegradable matrices can also provoke thrombus formation [23–26]. Additional modification

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of the surface of tissue-engineered highly porous vascular grafts with antiplatelet and anticoagulant drugs, which can prevent thrombotic processes after implantation of such grafts into the vascular bed, can be a solution. Additional introduction of bioactive substances such as growth factors, chemokines, interleukins, amino acids and others into the prosthesis structure and their prolonged release can imitate natural biochemical signals and guide the regeneration process with the formation of all structural layers of vascular tissue, including endothelium [27–29].

Thus, highly porous biodegradable constructs need additional modification of their surface with anti-thrombogenic substances in order to avoid the risk of failure after implantation of such a construct in the vascular bed [30, 31].

MATERIALS AND METHODS

Fabrication of vascular grafts

PHBV/PCL vascular grafts ∅4 mm in diameter and 40.0 mm long were fabricated by two-phase electrospinning from 5% PHBV (Sigma-Aldrich, USA) and 10% PCL (Sigma-Aldrich, USA) on a Nanon-01A apparatus (MECC, Japan). Chemically pure chloroform (Vekton, Russia) was used as a solvent.

To incorporate the growth factor and chemoattractant molecules into the polymer fiber, a PHBV/PCL chloroform solution was thoroughly mixed with a 20:1 solution of one or more differentiation factors diluted in phosphate-buffered saline (PBS; Gibco, USA) to form a suspension. One-third of the inner part of the prosthetic wall was made from PHBV/PCL solution, with the addition of human vascular endothelial growth factor (VEGF; Sigma-Aldrich, USA). The outer two-third part of the prosthetic wall was made from PHBV/PCL solution with a mixture of recombinant human basic fibroblast growth factor (bFGF; Sigma-Aldrich, USA) and recombinant human chemoattractant molecule, stromal cell-derived factor-1α (SDF-1α; Sigma-Aldrich, USA).

Formation of an anti-thrombogenic coating on the surface of biodegradable vascular grafts

The surface of the PHBV/PCL/GFmix graft was additionally modified with antiplatelet agents and anticoagulants according to our own original method to increase thromboresistance [32].

To modify the internal surface of the graft, 10.0% solution of PVP (PanReac, Germany) was prepared in ethanol. The prosthesis was immersed in the PVP solution for 30 minutes, filling its internal canal completely with the solution. The prosthesis was then removed from the solution and dried horizontally for 24 hours.

To graft PVP to the surface of the polymer prosthesis, the product was placed in a glass tube that was filled with inert argon gas and irradiated with ionizing radiation with 15 kGy total absorbed dose.

Before drug adhesion, non-grafted PVP residues were three times washed off the surface of the vascular graft placed in tubes with sterile water for injection. Each wash lasted for 30 minutes.

A modifying solution consisting of glycine buffer solution (pH = 2.5–2.6) with the anticoagulant Hep (Diamed-Pharma, Russia) at 5000 IU/ml concentration and antiplatelet agent Ilo (Bayer, Germany) at 0.2 μg/ml concentration was prepared in sterile conditions. To attach the drugs to the remaining free reactive groups of the grafted PVP, the vascular grafts were incubated in the modifying solution for 30 minutes. Then, they were air-dried under sterile conditions for 24 hours.

Assessment of physical and mechanical properties

The mechanical properties of PHBV/PCL biodegradable drug-coated vascular grafts before and after formation of an additional anti-thrombogenic drug coating were evaluated under uniaxial tension conditions on a Z-series universal testing machine (Zwick/Roell). The ultimate tensile strength of the material was estimated as the maximum tensile stress (MPa) before failure. The stress-strain properties of the material were evaluated by relative elongation to failure (%) and Young's modulus (MPa). Synthetic Gore-Tex graft (ST04010A, USA), native human internal thoracic artery (a. mammaria), and sheep carotid artery were used as controls.

Scanning electron microscopy

The surface structure of biodegradable PHBV/PCL/GFmix vascular grafts before and after formation of anti-thrombogenic drug coating, as well as synthetic Gore-Tex vascular grafts was assessed on scanning electron microscope S-3400N (Hitachi, Japan) under high vacuum at 10 kV accelerating voltage. Before the study, 0.5 × 0.5 cm prosthesis samples were subjected to gold-palladium sputtering to obtain a 15 nm thick coating using sputtering system EM ACE200 (Leica Mikrosysteme GmbH, Austria).

Implantation of vascular grafts into the carotid artery of sheep

All groups of vascular grafts were implanted into the carotid artery of Edilbay sheep. The experimental group of PHBV/PCL/GFmix$^{Hep/Ilo}$ prostheses (n = 8) was implanted for 18 months.

Synthetic Gore-Tex® grafts (Number ST04010A, USA), (n = 5) implanted in the carotid artery of sheep for 6 months (taking into account their early thrombosis 1 day after implantation) were used as the comparison group.

Anaesthetic support

Premedication: xylazine (Xylanit) 0.05–0.25 ml per 10 kg of animal weight + atropine 1 mg intramuscularly. Anesthesia induction: 5–7 mg of propofol per 1 kg of animal weight, within 90 seconds after – atracurium besylate (Ridelat) was administered intravenously in a 0.5–0.6 mg/kg dose. Tracheal intubation with a 9.0 diameter endotracheal tube. Anesthesia maintenance: Sevoran 2–4 vol%, continuous infusion of Ridelat at 03–0.6 mg/kg/h.

Main stage of vascular graft implantation

Access to the carotid artery; systemic heparinization – 5000 units administered intravenously; carotid artery clamping, resection of the isolated segment at a 45-degree angle, end-to-end implantation of vascular grafts with continuous twist suture proximally and then distally with prolene 6/0 sutures (Ethicon, USA). Standard protocol for prevention of air embolism and triggering of blood flow; wound closure with Vicril 2.0 suture (Ethicon, USA); suture treatment with butyral phenolic adhesive, sodium enoxaparin subcutaneously 4,000 IU anti-Xa/0.4 ml; extubation.

Intraoperative drug administration: infusion of 0.9% NaCl 500 mL – IV drip; Axetine (cefuroxime) 1.5 g – IV drip; Postoperative drug therapy: antibiotic therapy (Axetine (cefuroxime) 1.5 g – intramuscular twice/day + Enoxaparin sodium subcutaneously 4,000 IU anti-Xa/0.4 ml for 5 days. With proven patency of biodegradable prostheses: clopidogrel 75 mg orally once/day + sodium heparin 5000 units subcutaneously twice/day) – for 1 month.

Postoperative ultrasound screening of patency in implanted vascular grafts: for permeable prostheses – day 1 and 5, then once every 3 months up to the estimated date of animal's withdrawal from the experiment; for thrombosed grafts – day 1 and 5.

Histopathology

Explanted prosthetic specimens were subjected to histological examination with H&E stain, Van Gieson's stain, orcein stain, and alizarin red S stain.

The explanted specimens were fixed in formalin for 24 hours, then washed with running tap water to remove the fixative solution, and dehydrated in IsoPrep (BioVitrum, Russia). Samples were impregnated with paraffin (3 portions) at 56 °C for 60 minutes in each portion. Impregnated samples were filled with Histomix paraffin (BioVitrum, Russia). From the obtained samples, 8 μm thick sections were made using an HM 325 rotary microtome (Thermo Scientific, USA). The samples were then placed in an oven and dried overnight at 37 °C. After complete drying, samples were deparaffinized in o-xylene (3 portions) for 1–2 minutes and dehydrated in 96% alcohol (3 portions) for 1–2 minutes. The deparaffinized sections were then stained according to the staining protocol. The samples were examined by light and fluorescence microscopy using an AXIO Imager A1 microscope (Carl Zeiss, Germany) with 50×, 100×, and 200× magnifications.

Confocal fluorescence microscopy

From frozen explanted specimens, 8-μm-thick sections were made using a cryotome (Microm HM 525, Thermo Scientific).

The sections were fixed in 4% paraformaldehyde solution for 10 minutes.

Before staining for intracellular markers, the sections were permeabilized with Triton-X100 solution (Sigma-Aldrich, USA) for 15 minutes. The prepared sections were stained using specific primary antibodies: rabbit anti-CD31 antibodies (Abcam, UK) and mouse alpha smooth muscle actin antibodies (α-SMA, Abcam, UK); rabbit antibodies to von Willebrand factor (vWF, Abcam, UK); rabbit antibodies to collagen type IV (Abcam, UK) and mouse collagen type I antibodies (Abcam, UK); rabbit collagen type III antibodies (Novus Biologicals, USA). The sections were incubated with primary antibodies overnight at 4 °C, then with secondary donkey anti-mouse IgG antibody conjugated with Alexa Fluor 488-conjugated (Thermo Fisher, USA) and donkey anti-mouse IgG antibody conjugated with Alexa Fluor 555-conjugated (Thermo Fisher Scientific, USA) for 1 hour at room temperature. At all staining stages, phosphate-buffered saline with 0.1% Tween (Sigma-Aldrich, USA) was used for intermediate washing of the sections. To remove autofluorescence, the sections were treated with Autofluorescence Eliminator Reagent (Millipore, USA) according to the manufacturer's procedure. Nuclei were contrasted using DAPI stain (10 μg/mL, Sigma-Aldrich, USA) for 30 minutes. The preparations were analyzed using a confocal laser scanning microscope LSM 700 (Carl Zeiss, Germany).

Examination of explanted vascular graft samples by SEM according to the original technique

The explanted samples were fixed in formalin for 24 hours, then postfixed with 1% osmium tetroxide in 0.1 M phosphate buffer and stained with 2% osmium tetroxide in double distilled water for 48 hours. The samples were then dehydrated in a series of alcohols of increasing concentration, stained with 2% uranyl acetate (Electron Microscopy Sciences, USA) in 95% ethanol, dehydrated with 99.7% isopropanol (BioVitrum, Russia) for 5 hours and with acetone (Reachim, Russia) for 1 hour, impregnated with a mixture of acetone and Epon epoxy resin (Electron Microscopy Sciences, USA) in a 1:1 ratio (6 hours), then transferred to a fresh portion of epoxy resin (for 24 hours) and further polymerized

in FixiForm containers (Electron Microscopy Sciences, USA) at 60 °C. After that, the samples in epoxy blocks were grinded and polished on a TegraPol-11 machine (Struers, USA). Lead citrate contrasting was performed by Reynolds's stain for 7 minutes by applying the solution to the surface of the ground sample followed by washing with double distilled water. Next, epoxy carbon blocks (10–15 nm coating thickness) were sprayed on the polished surface using a vacuum sputtering station (EM ACE200, Leica). The structure of the samples was visualized by scanning electron microscopy in backscattered electrons using a Hitachi-S-3400N electron microscope (Hitachi, Japan) in the BSECOMP mode at 10 kV accelerating voltage.

Examination of the elemental composition of explanted vascular graft samples

To assess the elemental composition of the examined samples, we used X-ray spectral microanalysis performed using XFlash 4010 energy dispersive spectrometer (Bruker), which is a part of S-3400N scanning electron microscope (Hitachi). Elemental analysis was carried out under conditions of low vacuum (20 Pa pressure in the microscope chamber) and at 15 kV accelerating voltage in a scanning electron microscopy mode in backscattered electrons, without using standard samples. Macrophages, giant multinucleated cells, neutrophils, smooth muscle and mast cells were identified on digital photomicrographs, their localization and interaction between themselves and with other vascular graft elements were determined.

Comparative assessment of gene expression profile characteristic of native vascular tissue in the wall of the explanted vascular graft and native sheep carotid artery

To assess gene expression, the materials used were dissected sections of the vascular graft and carotid artery, as well as endothelial cell wash obtained by washing the vessels and prosthesis with lysis reagent TRIzol (Invitrogen, USA). The genes of interest were *IL1B, IL6, IL6, IL10, IL8, IL12A, TNF, VEGF, CXCR4, NR2F2, SNAI2, ICAM1, YAP1, IFNG, KDR, FGF2, MMP2*, and *TGFB*. Immediately after resection, a section of the vascular graft or carotid artery was placed in a test tube containing 900 μL of TRIzol (Invitrogen, USA) for further RNA isolation. Before starting the experiment, all work surfaces and laboratory equipment were treated with decontamination solution RNaseZapTM RNase (Invitrogen, USA). Samples were homogenized on an apparatus (MP Biomedicals, USA). RNA was isolated according to the standard protocol using the Chomczynski method (guanidinium thiocyanate-phenol-chloroform extraction). The amount and quality of isolated RNA were assessed using a NanoDropTM 2000 spectrophotometer (ThermoScientific, USA). RNA integrity was determined using a Qubit 4 Fluorometer spectrophotometer (Invitrogen, USA) by measuring the RIQ (RNA Integrity and Quality) index. A complementary DNA (cDNA) molecule was synthesized from 100 ng of isolated RNA using reverse transcription reaction and a commercial High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, USA). The quantity and quality of the synthesized cDNA were assessed using a NanoDropTM 2000 spectrophotometer (ThermoScientific, USA). Gene expression was assessed by quantitative polymerase chain reaction (qPCR) with real-time detection of amplification products with SYBR fluorescent dye on a ViiA 7 Real-Time PCR System amplifier (Applied Biosystems, USA). For each sample, 10 μl of reaction mixture was prepared, containing 5 μl of PowerUpTM SYBR® Green Master Mix (Applied Biosystems, USA), a mixture of forward and reverse primers at 500 nM final concentration, and 10 ng of cDNA. PCR was performed in a 96-well optical plate containing, in addition to the samples analyzed, five standards with double dilution and a negative control (reaction mixture without cDNA). Three technical replicates were prepared for each analyte, standard, and negative control. Amplification was performed according to the following scheme: 2 minutes at 50 °C, 2 minutes at 95 °C, 15 seconds at 95 °C, and 1 minute at 60 °C (40 cycles). Reaction specificity and efficiency were checked by analyzing melting curves and amplification plots in QuantStudioTM Real-Time PCR Software v.1.3 (Applied Biosystems, USA). PCR results were normalized using three reference genes ACTB, GAPDH, and B2M in accordance with available recommendations. Expression of the studied genes was calculated by the $2^{-\Delta\Delta\Delta C}{}_{T}$ method and expressed as a multiple change relative to control samples.

Statistical analysis

Study results were processed using GraphPad Prism (Graph Pad Software). Normality of distribution was assessed using the Kolmogorov–Smirnov test. Significance of differences between two independent groups was determined using nonparametric Mann–Whitney test. Nonparametric Kruskel–Wallis analysis of variance was used to compare three or more independent groups; Dunn's test was used for pairwise comparison of groups. Differences were considered statistically significant at $p < 0.05$. Data are presented as mean and standard deviation M ± SD, as well as median and 25th and 75th percentiles Me (25%; 75%).

RESULTS

Study of mechanical properties

The study of physical and mechanical properties revealed a statistically significant increase in the stiffness of PHBV/PCL/GFmix vascular grafts after modification with PVP and subsequent complexation with Hep and

Ilo (Table). The Young's modulus values of PHBV/PCL/GFmix$^{Hep/Ilo}$ grafts were 5.8 times higher than that of PHBV/PCL/GFmix and 20.6 times higher than that of a. mammaria ($p < 0.05$), and that of sheep carotid artery was 100 times higher. Also, biodegradable PHBV/PCL/GFmix$^{Hep/Ilo}$ drug-coated vascular grafts had the maximum force applied to the specimen before its destruction (Table). The relative elongation of PHBV/PCL/GFmix and PHBV/PCL/GFmix$^{Hep/Ilo}$ vascular grafts exceeded that of a. mammaria almost 4-fold ($p < 0.05$); for carotid artery, there was a 0.3-fold decrease. There were no significant differences between a. mammaria and polymer vascular grafts in terms of tension. Gore-Tex® synthetic grafts had good elastic properties similar to those of the native vessel, but at the same time had great strength. Therefore, the force applied to Gore-Tex® grafts before their destruction was more than 6 times greater than that of biodegradable prostheses and 22.9 times greater than that of a. mammaria ($p < 0.05$). The increased stiffness of the PHBV/PCL/GFmix$^{Hep/Ilo}$ drug-coated vascular grafts is probably due to graft surface polymerization with PVP and exposure to ionizing radiation.

Scanning electron microscopy of vascular grafts before and after surface modification with drugs

The surface of PHBV/PCL/GFmix grafts was modified with antithrombotic drugs by forming a PVP hydrogel coating on its inner surface, which is able not only to bind drugs as a result of complexation, but also temporarily (until its complete resorption) occupy the pore cavity, thereby reducing the risk of platelet adhesion to the prosthesis surface after implantation in the vascular bed. Besides, PVP's well-known hydrophilicity helps to reduce the degree of adhesion of protein molecules and blood cells, in particular, platelets, as well as prevent conformational changes in protein structures. Mobility of macromolecular chains in hydrogels, among other things, is due to the high desorption rate of protein molecules, which complements the spectrum of reasons for their anti-thrombogenic potential [33, 34]. The PHBV/PCL/GFmix$^{Hep/Ilo}$ grafts consisted of chaotically arranged microsized polymer fibers, 1.47 ± 0.67 μm in diameter (Fig. 1), forming micropores during their interlacing. Fig. 1 shows that after washing off the residual unpolymerized PVP from the graft surface and subsequent Hep and Ilo attachment to the remaining free reactive groups of PVP, the initial architectonics of the polymer matrix surface was preserved, and the water-soluble polymer covered only the surface of threads forming the tubular framework, without changing the appearance of micropores. The formed drug coating was sufficient to significantly improve the hemocompatibility properties of the graft surface, which had been previously proven in in vitro experiments: the maximum platelet aggregation after contact with graft surface with drug-coated PHBV/PCL/GFmix$^{Hep/Ilo}$ decreased by 2.1 times compared to analogous prostheses without drug-coated PHBV/PCL/GFmix. Against this background, platelet deformation index after contact with the surfaces of PHBV/PCL/GFmix$^{Hep/Ilo}$ and PHBV/PCL/GFmix grafts was 0 and 2.7, respectively [35].

The inner surface of Gore-Tex® synthetic grafts is represented by monolithic polymer fragments (Fig. 1), alternating with porous structures. The pores on the inner surface of Gore-Tex® are larger than those on the surface of PHBV/PCL/GFmix$^{Hep/Ilo}$.

Vascular graft implantation outcomes

A sheep model was used for preclinical testing of the developed grafts. It is the model of choice for in vivo evaluation of the effectiveness of cardiovascular implants [36]. It is believed that sheep are suitable for "worst-case modeling" due to the increased propensity of their vessels to thrombosis and calcification, which

Table

Mechanical properties of PHBV/PCL/GFmix polymer grafts before and after formation of antithrombogenic drug coating in comparison with Gore-Tex®, a. mammaria and sheep carotid artery

	Tension, MPa	Relative elongation, %	Young's modulus (E_{mod}), MPa
PHBV/PCL/GFmix (n = 9) M (25–75%)	3.045 (2.9; 3.2)$^{\&}$	121.7 (117.1; 129.6)$^{\#\,\&}$	8.6 (8.0; 9.64)$^{\#\,\&}$
PHBV/PCL/GFmix$^{Hep/Ilo}$ (n = 9) M (25–75%)	3.94 (3.78–3.99)$^{\&}$	109.17 (92.29–116.06)$^{\#\,\&}$	49.95 (44.9–54.7)*$^{\#\,\&}$
Gore-Tex® (n = 9) M (25–75%)	22.95 (22.42–23.47)**	337.0 (332.0–341.8)**	1.98 (1.36–2.59)
A. mammaria (n = 9) M (25–75%)	2.48 (1.36–3.25)$^{\&}$	29.72 (23.51–39.62)$^{\&}$	2.42 (1.87–3.19)
Sheep carotid artery	1.2 (1.06–1.9)	158.5 (126.0–169.5)	0.49 (0.39–0.66)

Note: *, $p < 0.05$ relative to PHBV/PCL/GFmix; $^{\#}$, $p < 0.05$ relative to a. mammaria; **, relative to all groups considered; $^{\&}$, relative to Gore-Tex®.

allows for the most rigorous testing of vascular grafts for their long-term patency and degeneration in vivo [36].

The need to form an anti-thrombogenic drug coating was down to the negative outcomes obtained earlier in the implantation of biodegradable PHBV/PCL/GFmix vascular prostheses into a sheep carotid artery [37]. High porosity of the wall resulted in early graft thrombosis in 100% of cases [37].

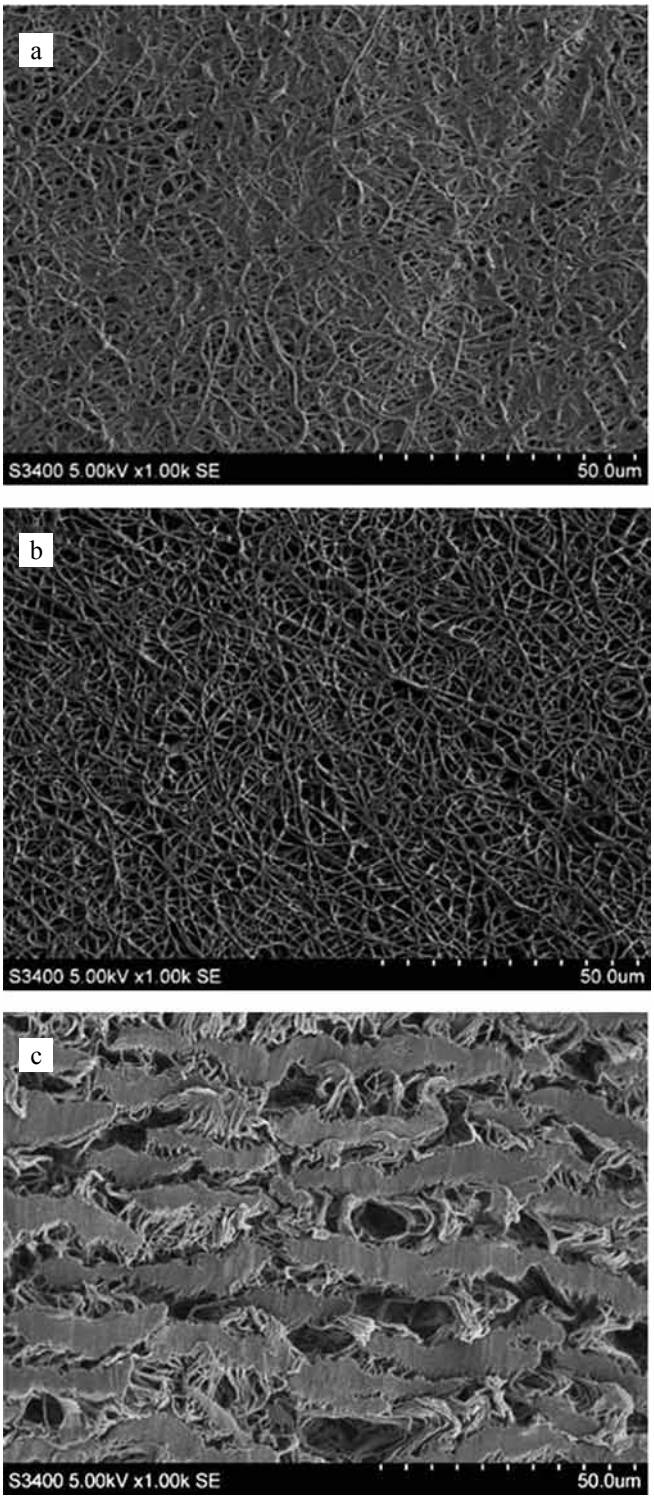

Fig. 1. Scanning electron microscopy of the inner surface of biodegradable and synthetic vascular grafts: a, inner surface of PHBV/PCL/GFmix$^{Hep/Ilo}$ graft before washing against PVP; b, inner surface of PHBV/PCL/GFmix$^{Hep/Ilo}$ graft after washing against PVP; c, inner surface of Gore-Tex® graft. 1000× magnification

In order to compare the effectiveness of the developed drug-coated vascular grafts with the grafts currently used in clinical practice, a comparison group of 4 mm-diameter synthetic Gore-Tex® grafts (Number ST04010A, USA) was also formed.

Patency of the PHBV/PCL/GFmix$^{Hep/Ilo}$ drug-coated grafts was 62.5% (5 of 8) 1 day after implantation in a sheep carotid artery (Fig. 2). After 18 months, patency of the PHBV/PCL/GFmix$^{Hep/Ilo}$ graft was 50.0%. However, all explanted prostheses that were permeable exhibited aneurysmal wall dilation throughout (Fig. 2).

One day after implantation of Gore-Tex® grafts, thrombosis was detected in 100.0% of cases (5 out of 5) (Fig. 2).

Outcomes of morphological study of explanted grafts

It was revealed that in place of the biodegradable vascular graft PHBV/PCL/GFmix$^{Hep/Ilo}$, a three-layer newly formed vessel similar in structure to native carotid artery was formed. However, the main difference between the newly formed vascular tissue and the native vessel tissue was aneurysmal dilation, absence of elastic fibers and clear elongation of smooth muscle cell cytoplasm, which is probably due to aneurysmal stretching of the formed vascular tissue under pulsatile blood flow conditions (Fig. 3). A small focus of coarse-grained calcium was detected in the thickness of only one PHBV/PCL/GFmix$^{Hep/Ilo}$ graft – between the media and adventitia (Fig. 3).

Recanalized thrombus was detected in the lumen of all explanted Gore-Tex® grafts 6 months after implantation. A thick connective tissue capsule was formed on the outside around the prosthesis. There was no formation of newly formed tissue in the graft thickness (Fig. 3). Despite the absence of blood flow, the Gore-Tex® graft underwent massive calcification (Fig. 3). Fine-grained calcium foci were also found in the outer connective tissue capsule. Nothing similar was found in the thrombosed PHBV/PCL/GFmix grafts implanted earlier in a sheep carotid artery for 12 months [37].

Examination of the explanted biodegradable PHBV/PCL/GFmixHep/Ilo drug-coated grafts by SEM revealed endothelial cells typical in morphology (Fig. 3). Examination of the thickness of the explanted prostheses showed that the newly formed vascular tissue that formed in place of the reabsorbed biodegradable tubular skeleton had three layers: neointima consisting of smooth muscle cells and covered by endothelium; the middle layer containing a large number of collagen fibers, fibroblast-like cells, macrophages, single giant multinucleated cells and vasa vasorum (Fig. 3). A small area of calcium deposition

was found at the junction between the neointima and the middle layer (Fig. 3). The middle layer was followed by the outer layer containing all elements typical for adventitia: vasa vasorum, single cells of a foreign body, perivascular fatty tissue, lymphoid follicles (Fig. 3).

A detailed layer-by-layer SEM study of the tissues formed around the explanted Gore-Tex® graft revealed that the entire lumen was filled with recanalized thrombus (Fig. 3). The prosthesis wall contained calcium inclusions, occupying 12–15% of the area (Fig. 3). Outside, the graft was surrounded by a layer of dense connective tissue consisting predominantly of fibrocytes and collagen fibers with a large number of newly formed vessels (Fig. 3). Calcium deposits were represented by shapeless heterogeneous formations often without clearly defined boundaries (Fig. 3). The internal structure of the calcifications was heterogeneous. In the deposit areas with minimal amounts of calcium, its presence was noted only in the outer part of the fibers forming the prosthesis (Fig. 3). With more pronounced calcification, calcium deposits were observed both in the outer structure of the fibers and in the space between them (Fig. 3). In the variant with maximum calcification, practically the entire space was filled with calcium deposits, inside which there were individual non-calcified fibers (Fig. 3).

Outcomes of confocal fluorescence microscopy

Immunofluorescence study of the explanted drug-coated grafts showed that the tissue formed in the place of the reabsorbed graft contained the main structural elements of the newly formed vessel: endothelial and smooth muscle layers were formed, a great number of collagens type I, III and IV was revealed. Collagen type IV was predominantly deposited in basal membrane, collagen type III – in basal membrane and graft wall, collagen type I – in adventitia (Fig. 4). The endothelial layer lining the neointima was represented by a double-row arrangement of endothelial cells active in terms of vWF synthesis, but simultaneously expressing CD31 and α-actin (Fig. 4). Such a picture may indirectly indicate the presence of endothelial-to-mesenchymal transition (EndMT), which can be triggered under conditions that are not physiological for the endothelium. In an aneurysmally dilated implant, turbulent blood flow may well be the cause capable of triggering the EndMT. Clusters of CD31 and vWF-positive endothelial cells were noted in the thickness of the prosthetic wall. Dense ordered tissue formed by actin-secreting cells with concentrically oriented clusters of vWF-secreting cells and collagen type III strands were found along the outer edge and outside of PHBV/PCL/GFmix$^{Hep/Ilo}$ graft (Fig. 4). It was revealed that the cells lining the neointimal surface

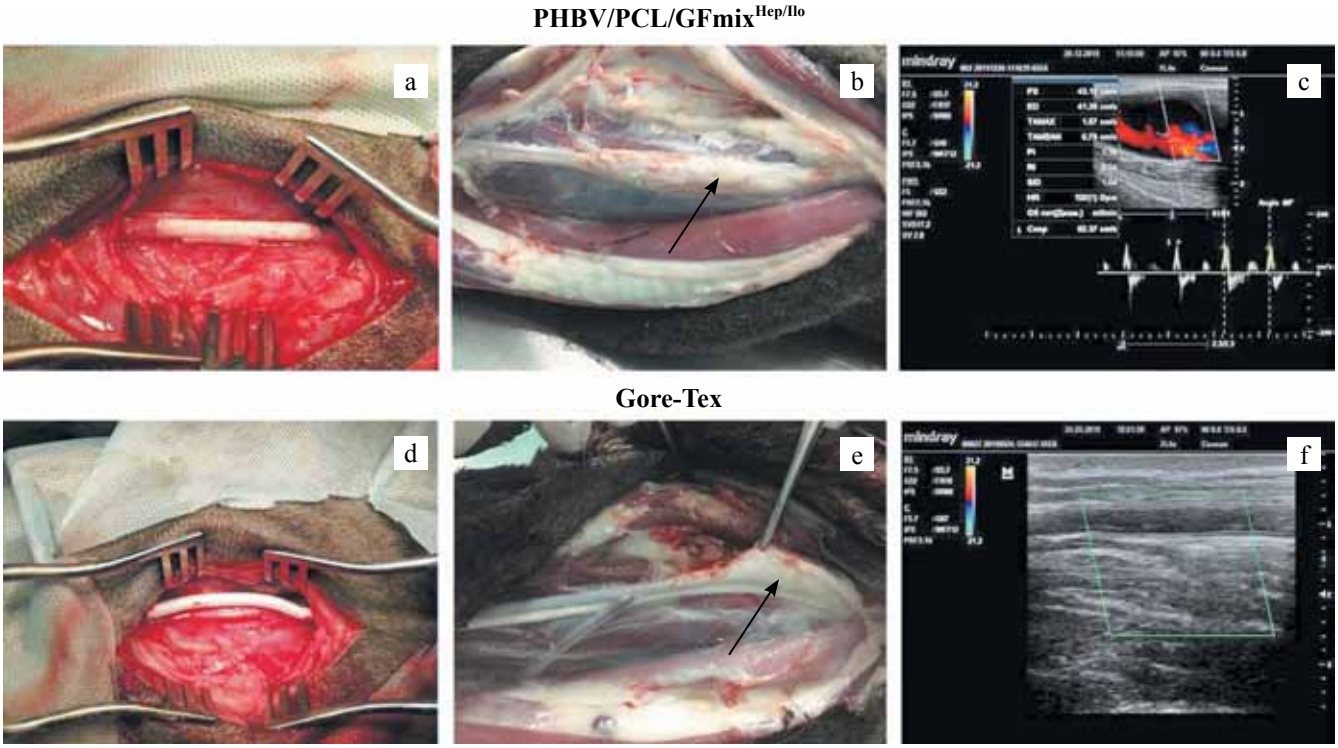

Fig. 2. Appearance and permeability analysis of PHBV/PCL/GFmix$^{Hep/Ilo}$ and Gore-Tex grafts: a, PHBV/PCL/GFmix$^{Hep/Ilo}$ implanted in the sheep carotid artery; b, PHBV/PCL/GFmix$^{Hep/Ilo}$ 18 months after implantation; c, ultrasound image of patency of PHBV/PCL/GFmix$^{Hep/Ilo}$ graft 18 months after implantation; d, Gore-Tex implanted in sheep carotid artery; e, Gore-Tex after 6 months of implantation; f, ultrasound image of Gore-Tex patency

in a monolayer from the vessel lumen side were mature endothelial cells synthesizing vWF, but with signs of EndMT (simultaneously expressing CD31 and α-actin). There was a basal membrane with collagen type IV. Collagen type III was found in the wall and in the basal membrane under the endothelial cell layer (Fig. 4). In the thickness of the explant wall and adventitial layer there was a large number of newly formed vessels and cellular elements (Fig. 4).

Immunofluorescence examination of synthetic Gore-Tex® vascular grafts after 6 months of implantation revealed an obstructive thrombus in the graft lumen;

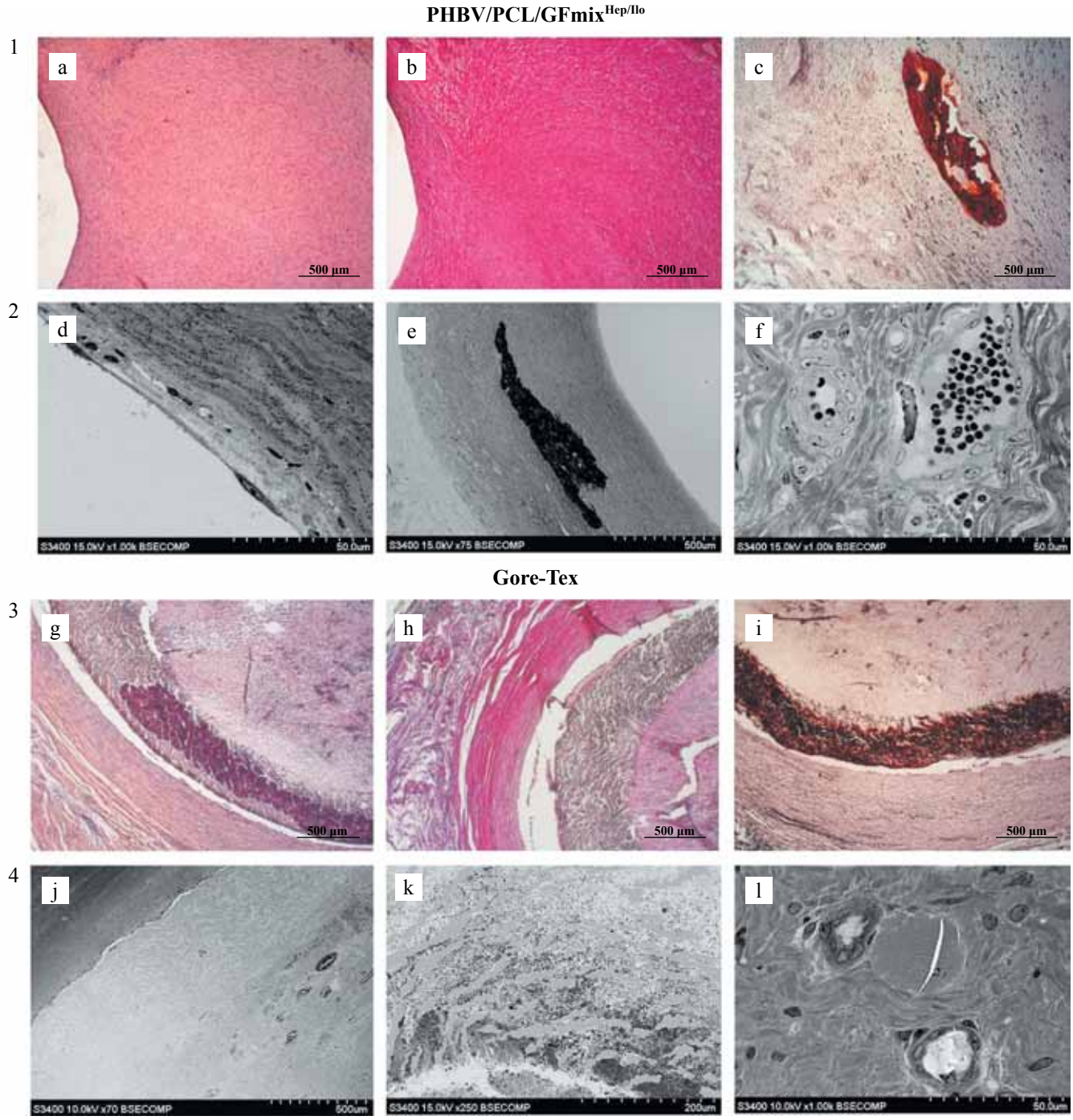

Fig. 3. Results of morphological study of explanted PHBV/PCL/GFmix$^{Hep/Ilo}$ vascular grafts 18 months after implantation: 1, histological study (a, H&E stain; b, Van Gieson's stain; c, alizarin red S stain), 50× magnification; 2, scanning electron microscopy (d, endothelium on the inner surface of the graft, 1000× magnification; e, transverse section of the explanted graft wall with a calcification area, 75× magnification; f, vasa vasorum, smooth muscle fibers in the middle layer of the graft, 1000× magnification). Results of morphological examination of explanted Gore-Tex® vascular grafts 6 months after implantation: 3, histological examination (g, H&E stain; h, Van Gieson's stain; i, alizarin red S stain), 50× magnification; 4, scanning electron microscopy (j, transverse section, 70× magnification; k, calcium in the graft wall thickness, 250× magnification; l, newly formed vessels in the outer sheath tissue, 1000× magnification)

no endothelial layer, neointima and media were found; no collagen type III was revealed, and only slight deposition of collagen type IV was found in the marginal zone on the inner lumen side (Fig. 4). Formation of connective tissue capsule with neorevascularization signs was detected on the outer surface of the explanted graft (Fig. 4).

Results of examination of the elemental composition of explanted vascular graft samples

A study of the elemental composition of solid calcium deposits showed their internal homogeneity in terms of their calcium and phosphorus content. The median value of the calcium to phosphorus ratio for various sites (n = 6) was 2.01, with a minimum of 1.96 and maximum of 2.05. In the calcium-containing areas, consisting predominantly of calcined fibers, the Ca/P ratio in the various fibers ranged from 1.2 to 2.32. It is likely that such differences in calcium content are due to the different calcification stages for specific fibers. In addition to biologically significant elements, about 2% fluorine was detected in the composition of prosthetic fibers, which indirectly indicates that the fibers themselves were intact at the initial calcification stages. In the areas with solid

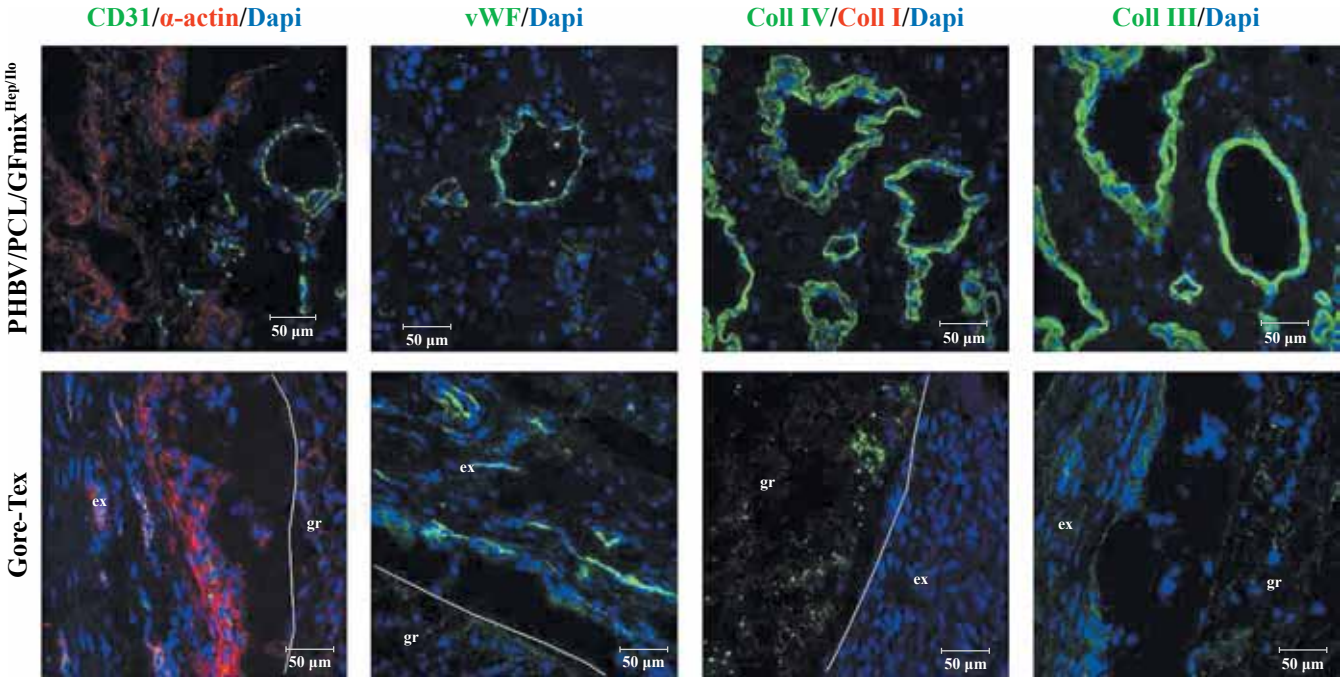

Fig. 4. Confocal microscopy of explanted vascular grafts PHBV/PCL/GFmix$^{Hep/Ilo}$ and Gore-Tex® staining with specific fluorescent anti-CD31 antibodies (mature endothelial cells), vWF (von Willebrand factor), α-actin (smooth muscle cell marker), collagen type I, collagen type III, collagen type IV, DAPI (fluorescent nuclear dye), 200× magnification

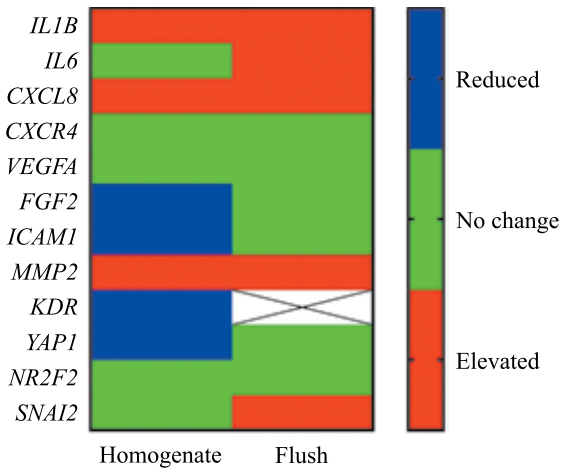

Fig. 5. Transcriptional profile of in situ regenerated PHBV/PCL/GFmix$^{Hep/Ilo}$ vascular grafts and intact contralateral carotid arteries 18 months after implantation

calcification inside the deposit, no fluoride was detected. This is probably due to the masking effect of the deposit itself, which reduces the accessibility of the probing electron beam inside the structure under study, rather than due to chemical destruction of the fibers.

Results of gene expression profile evaluation

The endothelial and wall transcription profiles of the regenerated artery were compared with those in the contralateral carotid arteries. Reverse transcription-quantitative polymerase chain reaction revealed an abundance of transcripts associated with inflammation (IL1B, IL6, and CXCL8), ECM remodeling (MMP2), and EndMT (SNAI2) in both RNA fractions obtained from the regenerated artery (Fig. 5). The endothelial lysate was enriched in inflammatory transcripts (IL1B, IL6, and ICAM1) and signs of endothelial reprogramming

(venous transcript NR2F2, and EndMT marker SNAI2) (Fig. 5). These observations suggest that the molecular landscape of the vascular tissue formed in place of the biodegradable vascular graft may differ from the corresponding blood vessels even during arterial regeneration in the long term (18 months after implantation).

CONCLUSION

The study on modification of vascular grafts made of PHBV/PCL polymer mixture using growth factors, as well as formation of a Hep + Ilo drug coating on the inner surface, attached through a PVP hydrogel coating by complexation method, showed successful creation of a highly porous and functionally active biodegradable vascular graft. It was on the basis of this graft that a newly formed vascular tissue similar in structure to the native carotid artery of a sheep can form over time. Also, the attachment of drug-containing hydrogel coating to the graft surface allowed to temporarily smooth the relief of the internal surface of the graft and enhance its anti-thrombogenic potential due to slow release of heparin and iloprost after implantation into the vascular bed. However, the presence of aneurysmal dilatation suggests that the newly formed tissue was incapable of resisting cyclic loads of blood flow. Therefore, despite the high biocompatibility obtained and the formation of newly-formed vascular tissue without initiation of inflammation and calcification processes, the outer frame of the developed design requires additional strengthening.

The research was done as part of a comprehensive scientific and technical program of a full innovation cycle "Development and introduction of a complex of technologies in the field of exploration and production of solid minerals, maintenance of industrial safety, bioremediation, creation of products of deep processing from coal raw materials, while consistently reducing the environmental impact and risks for the life of the population" (adopted by Order #1144r of the Government of the Russian Federation on May 11, 2022).

The authors declare no conflict of interest.

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BIOMARKERS OF RENAL TRANSPLANT FIBROSIS

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Fibrosis is one of the causes of kidney allograft loss, especially late after transplantation (up to 65% incidence after 2 years). The purpose of this literature review is to analyze studies examining noninvasive monitoring techniques for renal graft fibrosis.

Keywords: fibrosis, kidney transplantation, biomarkers.

Renal allograft fibrosis is a complex, dynamic and inevitable process that is the terminal stage of most progressive kidney transplant diseases. A number of studies have demonstrated that the progression of interstitial fibrosis is particularly noticeable in the first hours after transplantation (which may be a window for therapeutic intervention) and can be detected in kidney recipients even with good graft function [1]. Fibrosis can affect all parts of the kidney, namely the tubulointerstitium, the glomeruli (glomerulosclerosis) and the vessels (atherosclerosis and arteriolosclerosis).

In renal allografts, interstitial fibrosis and tubular atrophy are evaluated together because the two phenomena almost inevitably occur in parallel [2, 3]. Interstitial fibrosis and tubular atrophy (IF/TA) (hereafter referred to as renal allograft fibrosis) is detected in approximately 40% of renal allografts after 3–6 months and increases to approximately 65% of cases 2 years after transplantation; characterized by profound renal tissue remodeling, excessive formation/deposition of extracellular matrix fibrillar cells, which leads to impaired tissue architecture and microperfusion, which in turn reduces renal graft function [4]. In patients who return to dialysis therapy or require retransplantation, the most common cause of decreased allograft function is IF/TA, regardless of the primary cause of the transplanted kidney fibrosis. The degree of fibrosis affects kidney graft function and survival [5].

The clinical impact of IF/TA was first described in 2009 [6]. Several studies have highlighted the negative impact of this condition on major clinical outcomes, and it has also been suggested that IF/TA may be associated with inadequate immunosuppressive therapy and usually precedes chronic active T cell-mediated rejection [7].

New molecular and pathogenetic insights into the biological mechanisms associated with kidney graft fibrosis provide an opportunity to identify new potential biomarkers and select new, clinically valuable therapeutic targets, which is a major goal of research in nephrology and organ transplantation.

MECHANISM OF FIBROSIS

Native renal fibrosis and IF/TA in renal allografts probably have common mechanisms and pathophysiology of the process. However, development of fibrosis in the renal allograft is a multifactorial process and may be a consequence of pre-existing pathology of the donor organ, acute cellular, antibody-mediated (humoral) or mixed rejection crises, diabetes, ischemic and hypertensive damage to the graft, chronic nephrotoxicity, cytomegalovirus infection, and the number of biopsies performed on the renal transplant [3].

At the initial stage of the profibrotic process inside the graft, inflammation is initiated, which is an integral part of the body's defense mechanisms in response to damage. This phenomenon in the early stage of renal fibrosis is potentially reversible. However, if the fibrosis progresses, the extracellular matrix proteins undergo several biochemical modifications that make it irreversible [8].

The renin-angiotensin-aldosterone system (RAAS), hypoxia, acute cellular rejection and chronic inflammation, etc., are involved in the pathogenesis of IF/TA. Some of these pathways are partially induced by immunosuppressive therapy [9–12].

Tubular and glomerular cells produce proinflammatory cytokines depending on the etiology of kidney damage. In addition, inflammatory infiltrates (including neutrophils, macrophages, T cells and B cells) enhance the fibrotic process and, by activating endothelial cells of peritubular capillaries, can promote attraction of new interstitial mononuclear cells. Following neutrophils, macrophages infiltrate the damaged tissue, phagocytize and secrete fibrotic cytokines, leading to proliferation of fibroblasts and myofibroblasts, epithelial-mesenchymal

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transition (EMT) [5, 13], excessive accumulation of extracellular matrix (ECM) and pathological proteins not normally identified in renal tissue [3, 14]. Macrophages are the main source of transforming growth factor beta 1 (TGF-β1), a powerful chemoattractant for monocytes and macrophages, which play a major role in renal allograft fibrosis [15].

As reported by Toki et al. in protocol renal allograft biopsies one year after transplantation, macrophage infiltration at 1 year correlated with renal dysfunction at 1, 12 and 36 months posttransplant [16]. It is interesting to suggest that the assessment of macrophage infiltration at early renal transplant biopsy may have value for the subsequent prognosis of transplanted kidney function.

There are still debates about additional myofibroblast progenitor cells, including circulating cells originating from bone marrow, or about the transition from macrophages, epithelial or endothelial cells [17, 18]. In the kidney, EMT describes the transition and cellular migration of polarized epithelial tubule cells across the basal membrane to apolar mesenchymal cells in the interstitium. Mesenchymal cells can actively secrete components of extracellular matrix – collagens, fibronectin – which can contribute to scar formation [14, 19]. Evidence for EMT is convincing in studies conducted in vitro, but there is no such evidence in in vivo studies. Studies in rats have shown that EMT is involved in the development of IF/TA, and it correlates with increased oxidative stress. A correlation between EMT 3 months after kidney transplantation and late graft lesions expressed in IF/TA, observed 1 year after transplantation, has also been reported [9, 20, 21].

Other potential extracellular matrix-producing cells are fibrocytes, a multitude of circulating bone marrow monocytes with fibroblast-like properties, which, in the presence of profibrotic cytokines such as IL-4 and IL-13, differentiate and infiltrate the renal parenchyma and participate in fibrogenesis [8].

The term "oxidative stress" refers to the damage caused by the accumulation of reactive oxygen species in cells and tissues [22, 23]. During this condition, cells undergo profound functional and morphological changes: hyperexpression of mesenchymal markers (vimentin, smooth muscle alpha-actin, fibronectin), release of matrix metallopeptidase (MMP)-9 and -2, increased motility, decreased cytokeratin and E-cadherin levels and changes in heparan sulfate proteoglycans (HSPGs) [24, 25]. The most abundant HSPGs on renal tubular epithelial cells is syndecan-1, a factor that promotes renal tubule survival and repair after damage, and its level correlates with improved function of the injured kidney allograft. This factor appears to be regulated by several factors, including heparanase, endo-β-D-glucuronidase, which are involved in the pathogenesis of several renal diseases, especially in diabetic nephropathy, and are suggested to be involved in allograft pathology [26].

Kidney graft toxicity associated with immunosuppressive drugs, particularly calcineurin inhibitors, can provoke oxidative stress by disassociating the mitochondrial system of oxidative phosphorylation mediated by Ca^{++} increase [27, 28, 29]. Fibrotic changes that are secondary to these events can cause chronic graft hypoxia with activation of various biochemical mediators, including hypoxia-inducible factor, which activates a large number of target genes involved in the maintenance of homeostasis during hypoxia, such as vascular endothelial growth factor (VEGF), erythropoietin, epidermal growth factor receptor (EGFR) and platelet-derived growth factor (PDGF) [30].

All these events are accompanied by significant morphological changes (including architectural changes in the renal tubules, apoptosis, defects in cell cycle progression, microvascular rarefaction) leading to tubular atrophy, a condition that has ever been associated with allograft fibrosis [31, 32].

DIAGNOSIS OF RENAL ALLOGRAFT FIBROSIS

Instrumental methods. In renal allografts, ultrasonography and magnetic resonance imaging (MRI) are the two main instrumental methods for assessing fibrosis. There have been suggestions that ultrasound elastography for tissue elasticity estimation, an approach that has been relatively well established in assessing liver fibrosis, correlates with fibrosis in renal allografts, but several investigators have found no such correlation [33].

MRI-based elastography is an alternative approach, but the first results of a study of renal allografts with fibrosis by this method showed that renal tissue stiffness changes much less in fibrosis than in the liver, suggesting that elastography of transplanted kidneys may not be sensitive enough to assess fibrosis [34]. In the liver, tissue stiffness increases significantly with increasing fibrosis, whereas data on the stiffness and biomechanical properties of the kidneys at different degrees of fibrosis are lacking.

Pulsed-wave Doppler, in which a quantitative assessment of blood flow (absolute parameters: maximum systolic blood flow velocity and final minimum diastolic velocity; relative parameters: resistance index and pulse index) in vessels on the curve that is reflecting Doppler frequency shift spectrum, performed in the post-transplant period, is of great importance for prediction of renal transplant outcomes [35, 36]. In a study by Pykov M.I., it was shown that as IF/TA progressed, kidney graft function decreased, which was manifested in increased proteinuria, serum creatinine levels and decreased glomerular filtration rate ($p < 0.001$). At the same time, the more pronounced the fibrotic changes, the lower the peak systolic and end-diastolic velocities, resistance index and pulsatility index [37].

Morphological analysis methods. To date, the most accurate method of imaging and diagnosis of kidney graft pathology is punch biopsy. Even when clinical evaluation conclusively indicates the specific cause of allograft dysfunction, biopsy is still necessary to clarify the degree and severity of renal tissue damage and choose the most optimal treatment tactics [3, 20, 38]. In addition to biopsy "by indication", some centers perform biopsy "by protocol" to detect subclinical chronic conditions and track the progression of renal fibrosis, in particular, its quantitative assessment [5].

Servais et al. demonstrated that kidney transplant biopsies obtained at day 0, month 3 and month 12 showed a rapid progression of IF/TA from 19% to 27% at month 3 and 32% at month 12 after kidney transplantation [39]. Serum creatinine levels and glomerular filtration rate (GFR) played a limited clinical role in assessing histopathological changes in the graft.

A kidney biopsy is an invasive method of diagnosing graft pathology, and the procedure also requires hospitalization. Like any invasive procedure, kidney biopsy also has a number of complications; therefore, noninvasive, sensitive and etiologically specific biomarkers for the diagnosis of pathological processes in a transplanted kidney are essential [40].

BIOMARKERS OF TRANSPLANTED KIDNEY FIBROSIS

The ideal biomarker should be noninvasive, reflect the degree and dynamics of renal fibrosis treatment, and be more sensitive than established other diagnostic and imaging techniques [41, 42]. It is important to note that at present, none of the identified markers is specific for transplanted kidney fibrosis, but rather may reflect other processes occurring in the body [43].

Transforming growth factor beta (TGF-β) is a cytokine involved in the initiation of various cellular processes (regulation of cell proliferation, apoptosis, cell migration and differentiation, leads to the synthesis of extracellular matrix proteins by myofibroblasts) and is the main mediator of renal fibrosis due to the EMT signaling pathway activation [1, 30, 44]. One of the three main isoforms, TGF-β1, has the greatest biological and pathological effect [45].

A review of the literature on the pathogenetic significance of TGF-β in the development of renal fibrosis showed that TGF-β hyperactivation via signaling pathways occurs in renal tissue damage of various origins [23, 45, 46]. It is likely that TGF-β expression may have prognostic significance in assessing kidney transplant survival [47]. The TGF-β gene has a significant polymorphism, which presumably may be responsible for the genetically determined cytokine activity and its association with various diseases. A high-producing TGF-β1 genotype in combination with other cytokines is a risk factor for chronic graft nephropathy [48, 49].

It has been experimentally shown that anti-TGF-β therapy in rats reduces chronic rejection [50], and mycophenolic acid can inhibit allograft fibrosis by suppressing TGF-β effects [51]. TGF-β inhibition is not without potential serious side effects: firstly, TGF-β is a tumor suppressor, and its inhibition can accelerate tumor progression [52]. In vivo modulation of cyclosporine effects by altering TGF-β levels has been demonstrated to partially mediate the beneficial and undesirable effects of cyclosporine [53].

Galectin-3. The mechanism of action of galectin-3 (a family of beta-galactoside-binding proteins) may vary depending on its localization: inside the cell, it helps protect cells from apoptosis; outside the cell, its action, on the contrary, promotes cell death [54]. It has been established that at the site of damage, galectin-3 is secreted into the extracellular space, stimulating the process of fibrosis through activation and proliferation of resting fibroblast cells. There are new studies of the association of galectin-3 with kidney graft dysfunction in the long term after transplantation [55, 56]. Based on a retrospective analysis, it was shown that serum galectin-3 levels were elevated in kidney transplant recipients, and independently associated with increased risk of late graft failure; the results were independent of donor, recipient, and graft characteristics, including GFR [21]. Further studies are warranted to evaluate whether galectin-3-targeted therapy may represent a novel opportunity to decrease the high burden of late graft failure. Recipients with high galectin-3 levels, high systolic blood pressure (≥140 mmHg), and/or a history of smoking are at particularly high risk of kidney graft failure [21].

Platelet-derived growth factor (PDGF). In the PDGF family, three isoforms PDGF-B, -C and -D, as well as both receptors (a and b) are involved in the mechanisms of renal fibrosis [57, 58]. A study by E.M. Buhl et al. shows physiological PDGFR-β signaling in renal mesenchymal cells as important for normal renal development. PDGFR-β activation was sufficient to trigger progressive renal fibrosis, and this created a unique model to specifically study the effects, reversibility, and therapeutic interventions in renal fibrosis independent of inflammation, hypertension, or epithelial or endothelial damage [59].

Vascular endothelial growth factors (VEGF) are powerful angiogenic factors produced by macrophages, fibroblasts, hepatocytes, endothelial and other cells [60]. They participate in activation, proliferation, migration and differentiation of blood and lymphatic vessel endothelial cells by interacting with them through specific tyrosine kinase receptors [61].

Inflammation plays a crucial role in the initiation and development of renal fibrosis. Signal transduction via VEGF-C, VEGF-D, and VEGF receptor (VEGFR)-3 is a central molecular mechanism of lymphangiogenesis.

TGF-β induces peritoneal fibrosis in association with peritoneal dialysis and also induces peritoneal neoangiogenesis through interaction with VEGF-A. On the other hand, TGF-β has a direct inhibitory effect on the growth of lymphatic endothelial cells. Hiroshi Kinashi proposed a possible mechanism of the TGF-β/VEGF-C pathway in which TGF-β promotes VEGF-C production in tubular epithelial cells, macrophages, and mesothelial cells, leading to lymphangiogenesis in renal and peritoneal fibrosis. Connective tissue growth factor (CTGF) is also involved in fibrosis-associated renal lymphangiogenesis through interaction with VEGF-C, in part by mediating TGF-β signaling. Further clarification of the mechanism might lead to the development of new therapeutic strategies to treat fibrotic diseases [62].

Ying Zhang and colleagues suggested that there is a close relationship between macrophages and lymphatic endothelial progenitor cells in renal fibrosis. The study demonstrated that lymphangiogenesis was positively correlated with the degree of fibrosis and macrophage infiltration. Compared to resting (M0) macrophages and alternatively activated (M2) macrophages, classically activated (M1) macrophages predominantly transdifferentiated into lymphatic endothelial cells (LECs) in vivo and in vitro. VEGF-C further enhanced polarization and transdifferentiation of M1 macrophages into LECs by activating VEGFR3. It was suggested that VEGF-C/VEGFR3 pathway activation downregulates macrophage autophagy and subsequently regulates the macrophage phenotype. The induction of autophagy in macrophages by rapamycin decreased M1 macrophage polarization and differentiation into LECs. These results suggest that M1 macrophages promote lymphangiogenesis and contribute to newly formed lymphatic vessels in the renal fibrosis microenvironment [63].

MicroRNAs (miRNAs). A separate group of signaling molecules considered as promising candidates for the role of biomarkers of post-transplant complications in kidney transplant recipients are miRNAs, small noncoding RNAs (18 to 25 nucleotides) that regulate gene expression and play an important role in regulating the functions of both healthy and damaged cells [64, 65]. Currently, very few studies on the role and diagnostic significance of miRNAs in post-transplant complications in kidney recipients have been published. At the same time, new data on the functions of currently known microRNA molecules appear, for example, miR-144 demonstrates the involvement in the cascade of processes forming the syndrome of obliterating bronchiolitis in lung recipients [66]; increased miR-155 expression is associated with lung and kidney graft dysfunction [67, 68]; an association of miR-21, -122 levels in solid organ recipients with long-term graft outcomes has been shown [69].

Signaling molecules miR-21 [70], miR-214 [71] and miR-192 [72] have been shown to be profibrotic, whereas the miR-29 family [73], miR-200b [74] and miR-30e [75] are antifibrotic. It has been suggested that most miRs target TGF-β signaling to collagen expression or metabolic pathways. The TGF-β/Smad3 pathways play an important role in fibrosis. When nephrons are damaged, TGF-β signaling is activated, thereby stimulating the TGF-β1 receptor, which then activates the Smad3 pathway. In the context of renal fibrosis, Smad3 is pathogenic, whereas Smad7 is protective. MiR-433 is an important component of the TGF-β/Smad3 pathway, creates a positive feedback loop, and enhances TGF-β/Smad3 signaling. In vitro and in vivo expression of miR-433 regulates the development of fibrosis, which in turn is induced by TGF-β1, by enhancing the antizyme inhibitor Azin1, an important regulator of polyamine synthesis [76]. Chung et al. reported that miR-192 mediates TGF-β/Smad3-regulated renal fibrosis [72]. Further study of the biological functions of microRNAs and their expression profile is required for possible use in clinical practice as a potential predictor of complications.

CONCLUSION

The search for a non-invasive method of detecting fibrosis before the development of irreversible complications in a transplanted kidney is an important task in transplantology. Three potential biomarkers involved in the development of kidney transplant pathology – TGF-β, galectin-3 and microRNA – can be highlighted in the development of noninvasive diagnostic methods for allograft kidney fibrosis. They present new diagnostic opportunities and open up new therapeutic targets.

The authors declare no conflict of interest.

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EVOLUTION OF LIVER DONATION IN MOSCOW. MOVEMENT TOWARDS EXPANDED DONOR SELECTION CRITERIA

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The **objective** of the study was to investigate the evolution and trends in liver donation in the city of Moscow, with special focus on the expansion of liver donor selection criteria for transplantation. **Materials and methods.** The study included 1,548 effective donors (EDs) in the period from January 1, 2012 to December 31, 2020. Their basic characteristics – age and cause of death – are presented. The dynamics of changes in the age groups of donors and the dynamics of the number of ≥60-year-old liver donors were studied. The influence of expanded liver donor selection criteria over the dynamics of the number of transplant surgeries and patient flow on the waiting list was assessed. **Results.** During the study period, the number of effective liver donors (ELDs) in Moscow increased 4.7-fold. The average age of ELDs increased from 37.1 in 2012 to 48.8 in 2020. There was an absolute prevalence of donors who died from cerebrovascular accident compared with donors who died from traumatic causes, 83.4% vs 16.6%, respectively. Since 2016, there has been a progressive increase in ≥60-year-old liver donors; the number of such donors in 2020 reached 39, accounting for 13.6% of the total pool of EDs. The progressive growth in the number of liver transplants has significantly influenced patient movement on the waiting list. In 2012, there was a 25.2% increase in the number of liver transplants per 100 patients on the waiting list; by 2020, it had reached 86.6%. **Conclusion.** The results reasonably indicate an increase in liver donation and liver transplantation (LTx) in Moscow. Comparison of Russian data with those of leading foreign donor programs shows that the trends in the donor pool in the context of older age, including ≥60-year-old donors, and shifting causes of donor death towards cerebrovascular diseases are similar. An overall increase in donor activity and expansion of liver donor criteria contributed to an increase in the number of transplants performed per 100 patients on the waiting list, which, in turn, reduced the waiting time for a donor organ and increased the intensity of patient flow on the waiting list.

Keywords: effective liver donors, donor pool characteristics, expanded organ donation criteria.

INTRODUCTION

Today, LTx is considered the standard treatment for end-stage acute or chronic liver failure of all etiologies. More than 80,000 LTx procedures are performed worldwide every year. Survival rates are significantly better now than they were over the past 25 years, reaching 96% at 1 year and 71% at 10 years after transplantation, respectively. Among the most common nosologies in patients undergoing liver transplantation are cirrhosis (57.0%), primary and secondary (metastatic) liver cancers (15.0%), cholestatic liver diseases (10.0%), and acute liver failure (8.0%) [1, 2].

According to Eurotransplant, there were 1,481 people actively waiting for liver transplantation in 2020, and 2,446 new candidates were registered. During the same period, 1,323 deceased donor liver transplantations were performed [3]. The liver transplant waiting list in the Russian Federation in 2020 included 2,237 patients, and the number of transplants was 559 [4].

In Moscow in 2020, organ harvesting was performed in 263 EDs. Among the organs harvested were 187 livers used for transplantation (72.6%). Given the increase in the average age of effective organ donors, it is obvious that such a high proportion of donor liver explantation is associated with the expansion of liver donor selection criteria. The use of donors with extended criteria for liver transplantation is practically not studied in the publications of Russian authors. There are single publications, mainly devoted to the morphological assessment of the donor liver for the purpose of verifying its suitability for transplantation, without taking into account the donor's medical and epidemiological and clinical characteristics [5, 6]. The aim of this study is to investigate the evolution of liver donation in Moscow, the region with the highest donor activity in Russia, to analyze donors' medical and epidemiological characteristics and to identify donors that meet the expanded liver donation criteria as defined by leading world donor programs [1].

MATERIALS AND METHODS

The study included 1,548 EDs, of which 946 (61.1%) were ELDs in the period from January 1, 2012 to Decem-

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ber 31, 2020. We investigated the population characteristics of the ELDs – mean age, proportion (%) of nosological forms – causes of donor death, and the dynamics of donor age groups, taking into account the most common criterion for expanding the donor pool – ≥60-year-old donors. For the first time in population calculations, we used the number of transplants per 100 recipients of the waiting list and investigated its dynamics.

RESULTS AND DISCUSSION

Between 2012 and 2020, there was a 4.7-fold increase in the number of ELDs in Moscow [4, 7]. Comparison of the absolute number of ELDs and the ELDs/Eds ratio, expressed in %, shows that, from 2012 to 2016, the total number of Eds increased by 124.7%, while the share of donor pool utilization for liver transplantation was less than 60.0%, almost unchanged since 2012. From 2017 through 2019, a similar level of donor pool utilization for liver transplantation persisted, not exceeding 63.26% in 2019, despite a 39.3% increase in the total number of EDs over that period. It is only in the 1-year period from 2019 to 2020 that we see a 9.72% increase in liver transplant donor pool utilization activity, with a comparable 10.49% increase in liver utilization activity over the preceding 7 years (2012 to 2019) (Fig. 1). These data indicate that LTx programs in Moscow are slow in adopting the criteria for expansion of the suitability of donor livers for transplantation and, as a consequence, incomplete use of the progressively increasing donor resource.

According to the Spanish National Transplant Organization (NTO), over a similar time period, among all donors offered, both brain-dead (BD) and asystolic (AS) donors, the proportion of liver explants was at 90.0%, with the proportion of livers transplanted not exceeding 70.0% in a context of active growth of AS use observed since 2015 [8]. As presented in Fig. 1, the efficiency of donor pool utilization for liver transplantation in 2020 in Moscow was comparable to 73.46%. It is important to note that this level of efficiency in the use of donor resource for liver transplantation has been achieved for the first time in Moscow. In our opinion, this is due to the stability and efficiency of the organizational system of organ donation for transplantation in Moscow and the accumulation of necessary experience in liver transplantation programs required to work with expanded criteria donors.

Donor age is one of the conventional factors considered in the evaluation of both standard liver donors and expanded criteria donors. Apart from age, conventional factors in liver donor evaluation also include elevated levels of liver enzymes in the blood, hemodynamic instability, including circulatory arrest in brain-dead donors, a history of alcohol abuse, hypernatremia, and liver steatosis [9]. From the position of expansion of donor criteria for liver transplantation, the age of the donor is certainly an important factor. According to the data presented by the world's largest donor organizations, the age of liver donors has been gradually but steadily increasing over the years. In the United States in 1994, only 20% of liver donors were over 50 years old; today this figure has doubled [10]. The annual number of liver donors older than 65 years, according to UNOS, increased 14-fold in the United States from 1991 to 2001. According to ELTR (European Liver Transplant Registry), the proportion of ≥60-year-old donors increased 10-fold, from 2% to 20% [11]. The average age of liver donors in our study is also increasing, from 37.1 years in 2012 to 48.8 years in 2020 (Fig. 2). Analysis of foreign data presented above, shows that the increase in the average age of donors is determined by the number of ELDs over 60 years old. According to the NTO, the age com-

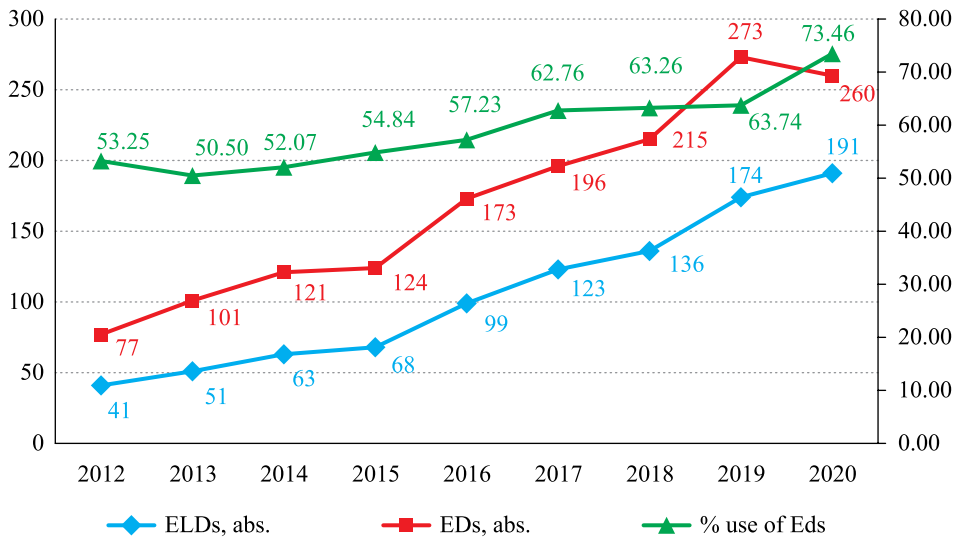

Fig. 1. Dynamics of the total number of effective donors (ED), total number of effective liver donors (ELD) and the ED/ELD ratio (%) in 2012–2020

position of the donor pool remained virtually unchanged from 2012 to 2020, with less than 10% of donors under 18 years and the 18–29 age range, 10–12% of donors aged 30–44 years, and quite equal shares of the so-called "older age" donors – 30.5% for age 45–59 years, 24.7% for age 60–69 years, 26.4% for donors ≥70 years [8].

Fig. 3 shows the percentage of the age groups of ELDs over the 2012–2020 period. Compared to the similar NTO data described above, the following age trends are evident in the Russian practice of liver donation – the youngest age group of donors (18–29 years) is progressively decreasing from 37.5% in 2012 to 6.3% in 2020; another group of young donors (30–39 years) for the entire study period remains fairly stable in the 20.0%–24.0% range; a similar stable situation is observed with the group of middle-aged donors 40–54 years, accounting for about 40.0%. The most significant changes are observed among the groups of older donors, contributing most significantly to the so-called "expanded donor criteria". The age 55–60-years-old group in our study went from 7.5% in 2012 to 24.1%. In 2012–2014, there were no ELDs in the 61–65 years age group; since 2015, their contribution to the ED pool has progressively increased, reaching 13.6% in 2020. The proportion of donors aged ≥65 years is still at a low level, up to 2.0% of the total ELD pool. Fig. 4 shows the evolution of the age of ELDs in Europe, according to the European Liver Transplant Registry. Noteworthy is the 30 years since the beginning of the work with donors in the ≥70 years age group, and more than 30 years of experience with donors in the 60–70 years age group. By 2019, the specific contributions of almost every age group except the youngest group 0–20 were comparable to each other, and the 60–70 and >70 age groups account for one-third of all ELDs. In other words, liver donors with expanded age criteria account for about 30.0% of the entire ELDs pool [12].

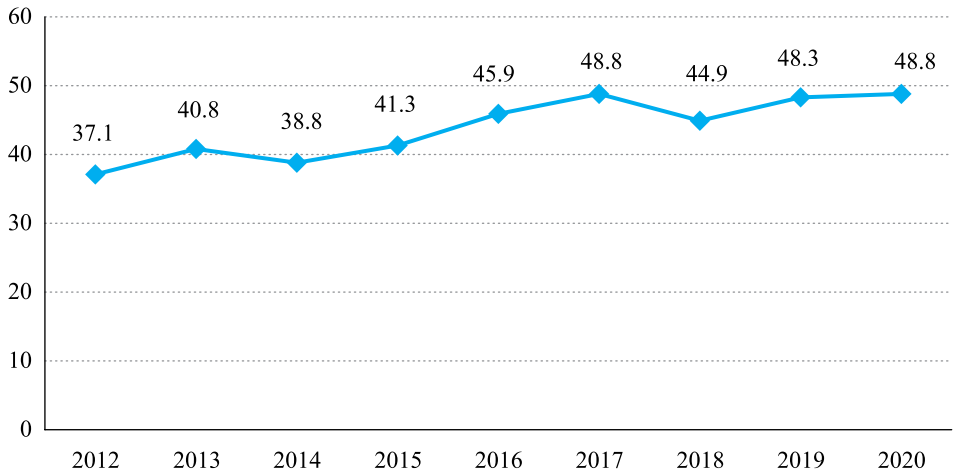

Fig. 2. Dynamics of the average ELD age in 2012–2020

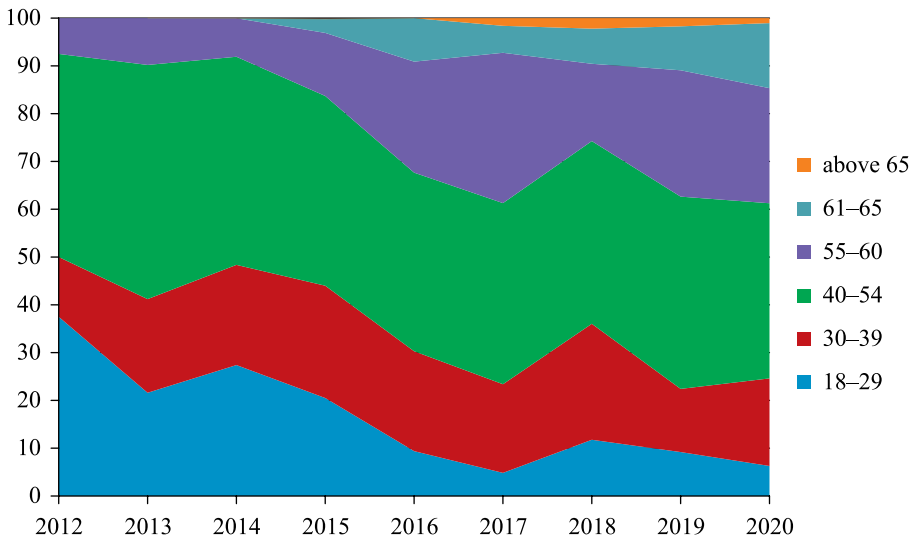

Fig. 3. Dynamics of the ELD age in 2012–2020

ELD age ≥60 years, according to the current selection criteria in Europe and the world, is classified as expanded [3]. In Moscow, there has been a progressive increase in the number of ELDs ≥60 years since 2016 (Fig. 5). The ratio of ELDs with expanded age criteria to the total number of ELDs shows that the proportion of expanded criteria donors is quite comparable with the foreign data given above, 20.4% in 2020. However, it is important to note that the number of liver donors in European countries also includes children, the 0–20-years-old age range, and people ≥70 years old.

Examination of the frequency of age distribution of ELDs in our study shows that 75.7% of expanded criteria donors are slightly older than 60 years, while there are single cases of liver donations from donors older than 65 years, and one case of liver donation was from a donor over 70 years old (Fig. 6). Of course, with the overall growth of donor activity and the accumulation of experience in liver transplantation programs, there is an obvious shift towards working with expanded criteria donors, but the age range of donors is practically not increasing, remaining within the 60–63-year range.

Along with the donor's age, the cause of death is of paramount medical and epidemiological importance. A number of papers presented by authors from the Moscow Organ Donation Coordination Center, Botkin City Clinical Hospital, and published in research journals have demonstrated serious changes observed in the structure of mortality in brain-dead donors in Moscow [7]. Obviously, these changes are also fully valid for ELDs. For instance, since 2015, there has been a progressive increase in the number of donors who died from acute stroke and there is an almost mirror-like decrease in the

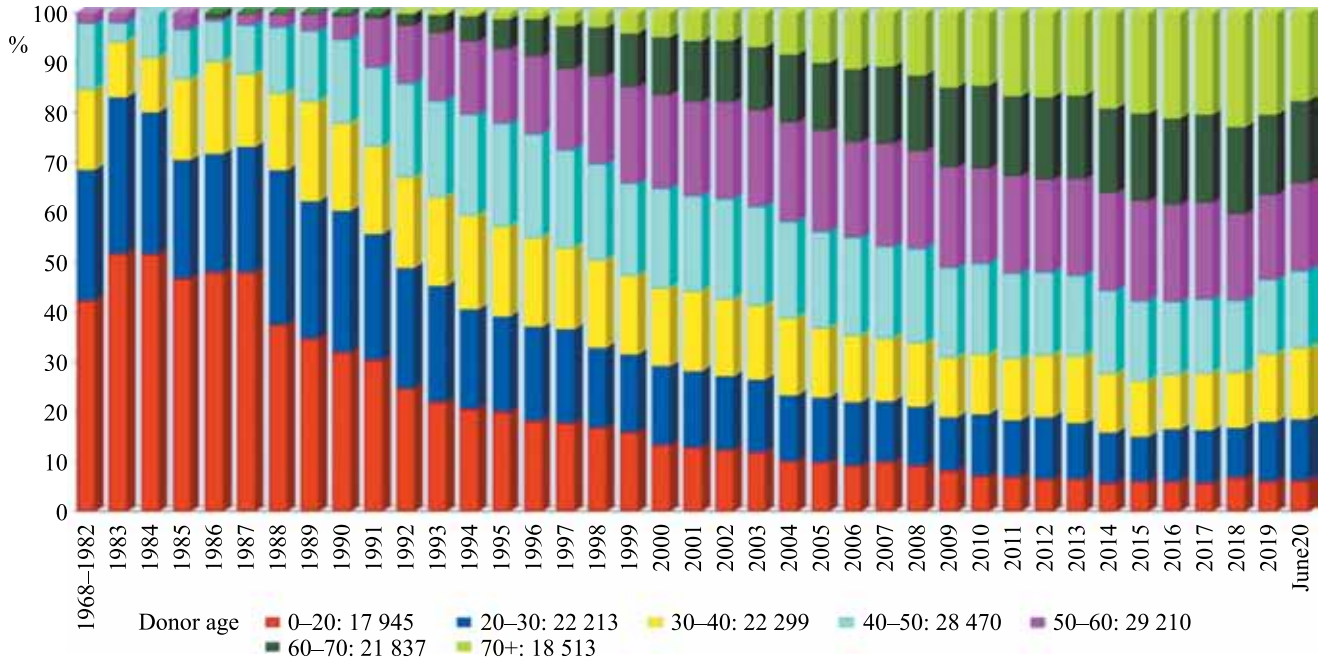

Fig. 4. Evolution of the ELD age in Europe according to the European Liver Transplant Registry (http://www.eltr.org)

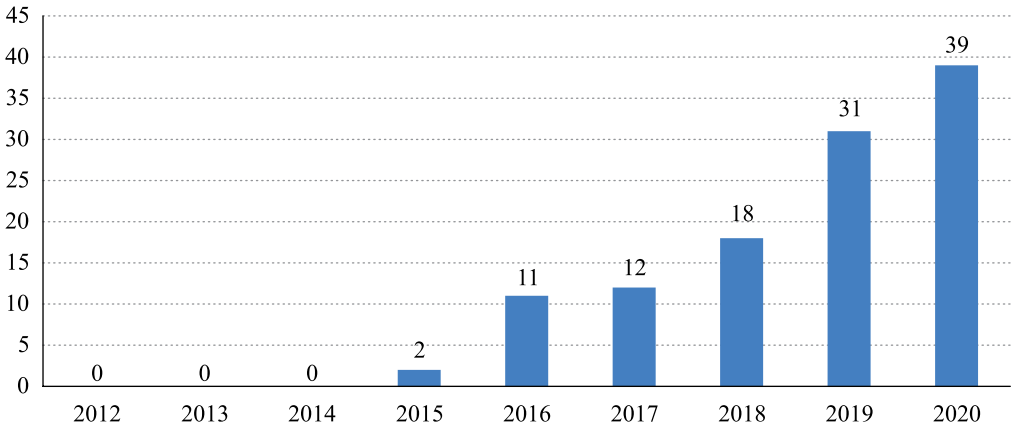

Fig. 5. The number of ≥60-year-old ELDs in 2012–2020

number of donors who died from traumatic brain injury (TBI). (Fig. 7).

Such significant changes in donor activity and donor mortality patterns could not but affect the evolution of liver donation in general and the development of donation according to expanded criteria. This is confirmed by the analysis of liver donors rejected for transplantation according to the age of the donor (Fig. 8). Against the background of some predominance of donors with TBI in the 2012–2014 period, we see a rather high proportion of liver rejections in the youngest age group of donors 18–29 years, accounting for 16.7% in 2012, and steadily decreasing to 4.1–6.4% only from 2016, when the number of TBI donors was sharply decreasing.

The most important social indicator of the effectiveness of organ transplantation is the movement of patients on the waiting list. Fig. 9 shows the dynamics of the indicator reflecting the number of liver transplantations per 100 patients on the waiting list per year. We can see that since 2016, after the expansion of liver donor selection criteria, this indicator begins to grow, indicating, on one hand, the effectiveness of the approach to the issue of selection in the context of increasing the number of transplants and the intensity of waitlist traffic. On the other hand, guided by the experience of foreign countries, where the same indicator reaches a maximum of 65, it indicates the need for further improvement of the waiting list with the formation of optimal routing of patients for inclusion in the waiting list (Fig. 9) [16].

CONCLUSION

The number of liver transplants in Moscow is increasing annually. At the beginning when the organizational structure of organ donation for transplantation was

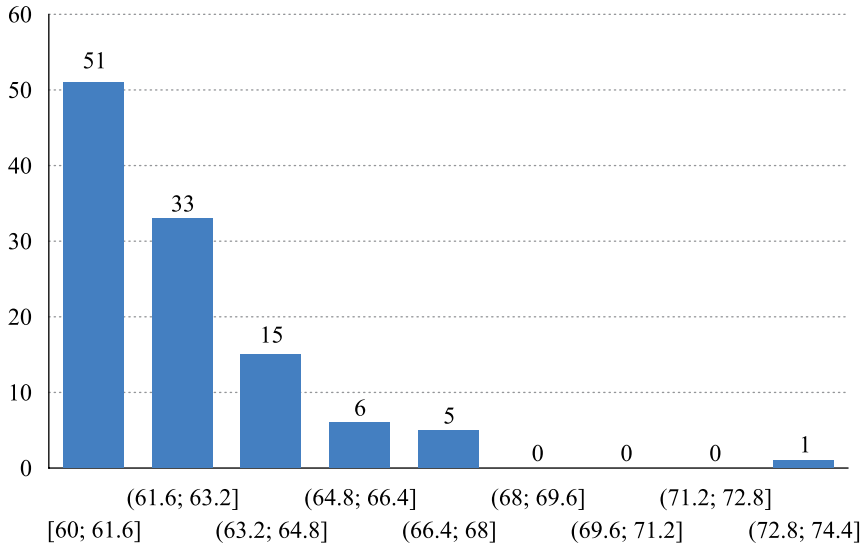

Fig. 6. Histogram of distribution of the frequency of liver donation from donors ≥60 years old in 2012–2020

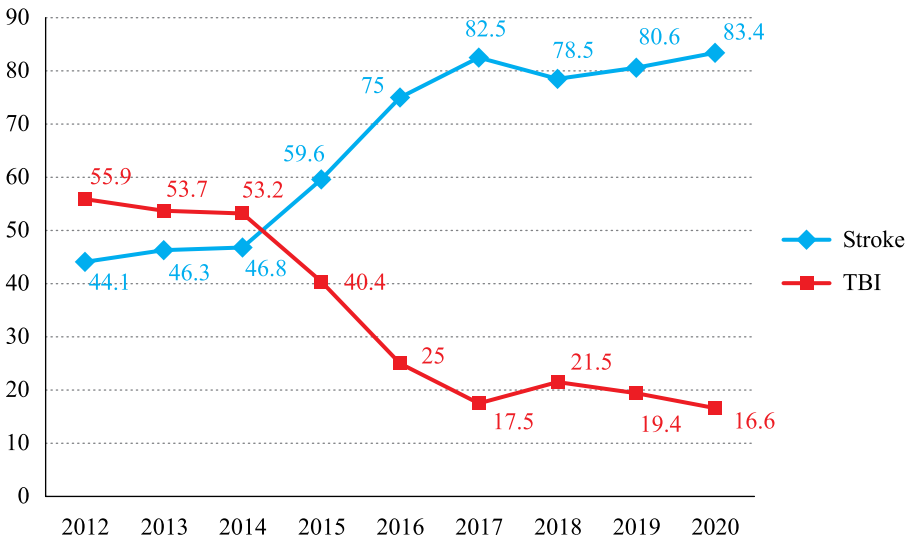

Fig. 7. Dynamics of ELDs depending on cause of death in 2012–2020 (%)

being formed in Moscow in 2012, there were 41 liver transplant surgeries per year, which was less than 20% of the need for this type of transplant care. In 2020, 191 liver transplants were performed in Moscow. Such a progressive increase in the number of transplants in a relatively short period of time was facilitated by a general increase in donor activity in Moscow as a result of implementation of the transplant coordination system, positive initial experience with expanded criteria donors, including donors ≥65 old, accumulated experience by transplant centers including with older donors, and increased number of liver transplant programs in the Moscow healthcare system.

To date, it is impossible to provide full-fledged transplantation care without working with expanded criteria donors. On one hand, the donor pool has significantly changed – the age of donors has increased and, accordingly, the incidence of comorbid diseases has increased; the main cause of donor death has become acute cerebral haemorrhage. On the other hand, the number of patients with end-stage chronic liver diseases is increasing and, accordingly, the number of donor organs suitable for transplantation needs to increase. Over the past 2 decades, the global transplant community has accepted the concept of expanded criteria donation. There is no universal definition of an expanded criteria organ donor; in general, it is a set of certain donor characteristics that can potentially increase the risk of organ transplantation for the recipient. For the liver, there are several lists of such donor characteristics, formulated by different authors in different years, with differences mainly affecting the "cut-off points", i.e. borderline values of certain indicators that allow the use of a liver for transplantation [17]. The most available indicator to estimate the volume of donation according to expanded criteria in the overall pool of effective donors is the age of the donor. For the

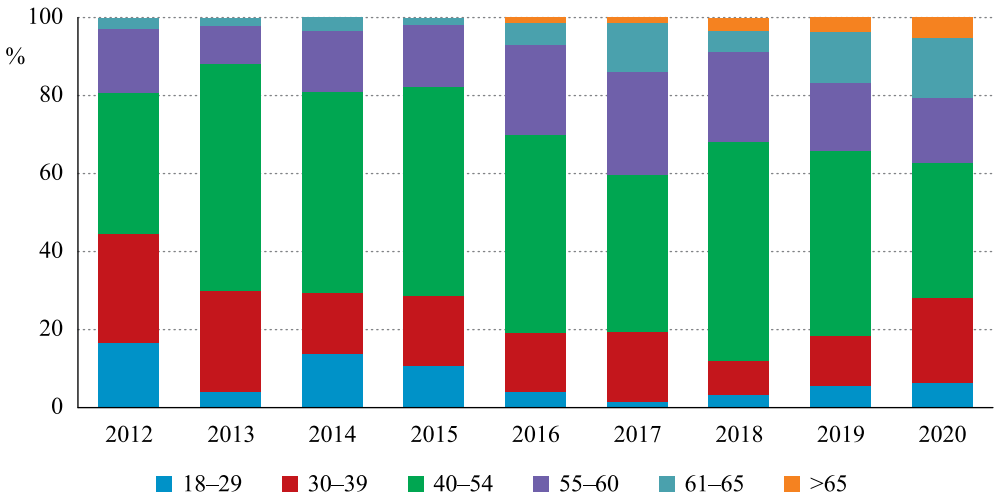

Fig. 8. Dynamics of the number of livers rejected for transplantation in different age groups of donors in 2012–2020 (%)

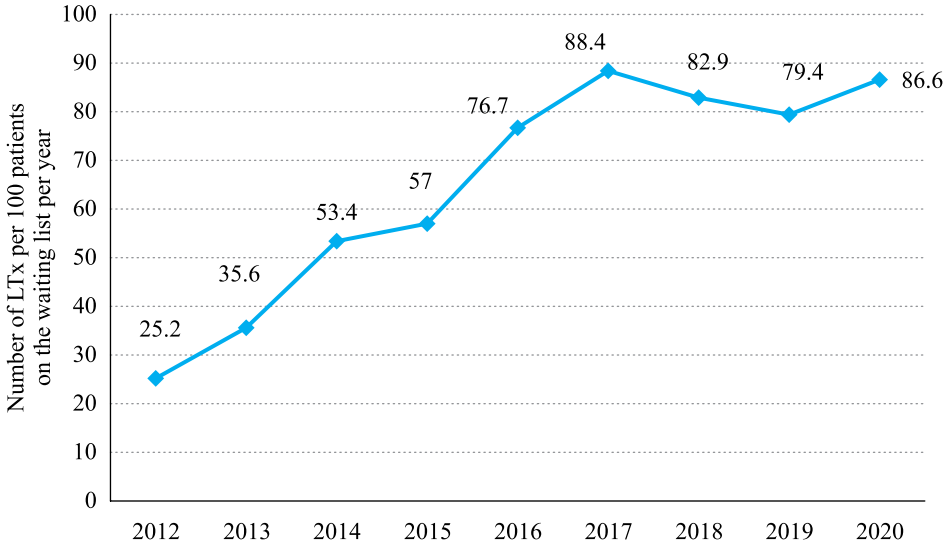

Fig. 9. Indicator reflecting the number of liver transplants per 100 patients on the waiting list per year

liver, the donor age defining a person as a standard or expanded criteria donor starts at 60 years of age, with no upper age limit defined; a number of authors indicate an age of up to 80 years [17].

Analysis presented in this study shows that there were liver donors over 60 years old in the donor pool in 2015; they are very few, 2 people, 2.9% of the total ED pool. Then the number of such donors increases every year, reaching 39 (13.6%) in 2020. At the same time, the proportion of organs rejected for transplantation increased; in 2020, 15.4% of livers were rejected for transplantation in this age group. In the vast majority of cases, the main reason for rejection was hepatic steatosis, detected both visually and by a "null" biopsy performed during the explantation procedure.

According to the largest foreign donor and transplant agencies, the 5-year survival rate of patients who received livers from donors 65 years and older is 74%, while the survival rate of recipients who received livers from younger donors was 75% [16].

Obviously, with comparable survival rates of recipients, the "60+" and "65+" donor categories can reasonably be used for transplantation, within the limits of acceptable safety for patients. It is important to note that a weighted and acceptable (for recipients) expansion of liver donation criteria is an effective organizational tool, allowing to reduce the waiting time for a transplant and to provide a more dynamic movement of patients on the waiting list.

In Moscow, the practice of using donors of the "60+" category has positive stable dynamics. Taking into consideration foreign and already available national experience, we consider it appropriate to develop and improve the engagement of expanded criteria donors in addition to the general development of organ donation for transplantation so that more effective transplant assistance could be provided to the Russian population.

The authors declare no conflict of interest.

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RESULTS OF A STUDY OF THE EFFECTIVENESS OF DIRECT CORONARY OXYGEN PERSUFFLATION AS A DONOR HEART CONDITIONING METHOD

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Objective: to evaluate the technical feasibility as well as functional, metabolic and structural integrity of donor heart myocardium after 4 hours of direct intracoronary oxygen persufflation in an experiment.**Materials and methods.** Mini-pig siblings aged 3 months with a body weight of 23–36 kg were used as the experimental model. In the control group (n = 8), donor hearts were cold preserved by injecting 2 liters of Bretschneider cardioplegic solution (Custodiol®, Germany, HTK) into the aortic root. In the experimental group (n = 8), modified HTK solution (with 40 mg/L hyaluronidase added) was used to initiate cardioplegia, then moistened carbogen (95% O_2, 5% CO_2) was injected into the ascending aorta, maintaining 40–45 mm Hg aortic root pressure. The hearts were stored in an mHTK solution at 0–4 °C. After 3 hours of donor heart preservation, orthotopic heart transplantation (OHTx) was performed. In the post-transplant period, we studied central hemodynamic parameters, myocardial oxygen consumption, level of myocardial ischemia markers (troponin I, TnI; creatine phosphokinase-MB, CPK-MB; lactate dehydrogenase, LDH), and histological signs of structural cellular injury. **Results.** Sixteen OHTx surgeries were performed during the study. At 120 minutes after restoration of spontaneous cardiac activity, cardiac output was 2.99 [4.85; 3.17] L/min and 2.48 [2.04; 2.92] L/min ($p > 0.05$) in the control and experimental groups, respectively. Changes in LDH, TnI and lactate levels in the blood flowing from the coronary sinus were significantly higher in the early reperfusion period. However, there was no statistically significant difference between the groups ($p > 0.05$). Myocardial oxygen consumption in the control and experimental groups was 8.2 [7.35; 9.35] ml-O_2/min/100 g and 7.7 [6.75; 10.12] ml-O_2/min/100 g, respectively ($p > 0.05$). Morphological examinations also showed no significant myocardial ischemia injury in the persufflation group compared to the control group. **Conclusion.** The experiment showed the technical feasibility and safety of direct intracoronary oxygen persufflation for 4 hours at the ex vivo donor heart conditioning stage. At the same time, experimental data showed no significant advantages of coronary persufflation over the standard protocol of cold preservation of donor heart with Bretschneider cardioplegic solution.

Keywords: oxygen persufflation, heart preservation, end-stage chronic heart failure, expanded donor criteria, cardiac output, heart transplantation, cold heart preservation.

INTRODUCTION

Organ shortage is largely determined by the geographical location of donor bases and transplant centers. The search for new strategies for prolonged conditioning of donor organs continues. As before, cold preservation of donor heart with the Bretschneider cardioplegic solution is the most frequently used method of transplant preservation in Russia and Europe. However, after four hours of preservation with Bretschneider, graft function can already be compromised, especially in elderly donors [1, 2]. This organ storage method is the greatest risk factor for primary allograft dysfunction and death [3]. Increasing cold ischemia time from 3 to 6 hours doubles the 1-year mortality after transplantation compared with a 50% reduction in predicted 1-year mortality if the ischemia period is less than 1 hour [4]. According to Kobashigawa J. et al., ischemia longer than 4 hours significantly increases the risk of primary graft dysfunction, which is associated with 8% 30-day mortality and increased mortality at 5 and 15 years after heart transplantation [5].

The optimal method of donor organ preservation includes three main aspects: hypothermia, composition of the preserving solution, and oxygenation [6]. If the first two conditions are fulfilled and can be corrected in any of the cold preservation methods, tissue enrichment with oxygen is associated with a number of problems. It has previously been shown that changing the formulation of the preservation solution (even with available ma-

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croergs and buffers) to remove metabolic waste had little effect on the functional outcome of transplantation, while the quality of oxygenation had a huge impact. Numerous variations of adjuvant cardioprotective prescriptions, including a wide range of pharmacological, metabolic and physical agents, have so far not resulted in any significant success [7].

Under natural conditions, the oxygen transporter substrate is blood hemoglobin, that is why the most physiological way of oxygen delivery to graft cardiomyocytes is continuous ex vivo perfusion of the graft with donor blood or macroergic substrate. The TransMedics system (Massachusetts, USA) is the first commercially available device for transporting a donor heart in a normothermic perfusion state. The perfusate is a proprietary infusion solution with the addition of insulin, antibiotic, methylprednisolone, sodium bicarbonate, multivitamins and fresh donor blood [8]. However, such methods are expensive and require constant monitoring, thereby complicating the organ transport stage [9–12].

In 1902, Rudolf Magnus made an unexpected observation while perfusing an isolated cat heart [13]. Despite the emptying of the reservoir storing liquid perfusate and pressurized air mixture delivery to the coronary channel, the heart continued to contract rhythmically for 9 minutes. In spite of a number of successes achieved in subsequent researches on cardiac preservation by feeding the oxygen mixture into the coronary channel (the term 'coronary oxygen persufflation' (COP) officially replaced 'gaseous oxygen perfusion' in 1971 [14]) interest in these works was reduced from 1960 till 1990s in favor of studies on liver and kidney perfusion [15]. However, after the 2000s, interest in long-term cardiac preservation by coronary persufflation was revived; the results of several studies proving the physiological possibility and efficiency of long-term (up to 14 hours) cardiac conditioning by persufflation, including after short (up to 16 minutes) periods of thermal ischemia have been published [15–18].

Not only the safety but also the very idea of performing PRA is still subjected to serious criticism by clinicians, despite the results of studies proving high efficiency of COP as a method of long-term graft conditioning. The purpose of this study was to technically adapt the COP technique to the current clinical protocol for OHTx and evaluate the effectiveness of this technique in comparison with the accepted cold heart preservation technique.

MATERIALS AND METHODS

Preparation of experimental animals, anesthesia

Piglets (mini-pigs) aged 3 months were used as the experimental model. Animal care, experiment support, observation and withdrawal of animals were performed in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasbourg, March 3, 1986). The study protocol was approved by the local bioethics committee, Meshalkin National Medical Research Center, Novosibirsk (protocol No. 1 of October 12, 2020).

On the day of implantation, all animals were premedicated on an empty stomach with a combination of atropine and zoletil-100. The dose was selected individually according to mass-growth parameters. After the onset of sleep, the surgical field and the neck vascular catheterization area were prepared. Then the animals were transported to the operating room and fixed in the supine position for subsequent tracheal intubation, placement of central arterial and venous catheters. The experiment was performed under endotracheal anesthesia with sevoflurane and myorelaxation (pipecuronium bromide). Artificial ventilation (AV) was maintained using anaesthesia workstation FabiusPlus (Dräger, Germany) with positive inhalation pressure (20–30 cm H_2O) and exhalation pressure (5–8 cm H_2O) with 8 mL/kg breathing volume and 12–14 breaths per minute frequency. During the experiment, we monitored invasive (intra-arterial) blood pressure (IBP) by catheterization of the left common carotid artery, central venous pressure (CVP) by catheterization of the right external jugular vein, heart rhythm disturbances (electrocardiography), body temperature, blood gas composition, and activated clotting time (ACT). Suprapubic cystostomy was implemented to monitor diuresis. Blood analysis was performed using automated hematology analyzer XT-4000i (Sysmex, Germany) according to the manufacturer's guidelines. Central hemodynamics parameters were studied by catheterization of the right heart with a Swan–Ganz catheter. The measurements were performed in the donor after anesthesia and beginning of AV, then after implantation of the donor heart into the recipient's body within two hours after the end of cardiopulmonary bypass according to the protocol (Fig. 1).

Vital parameters were recorded using an IntelliVue MP70 patient monitor (Philips, Netherlands). The study protocol included blood sampling from the coronary sinus to measure myocardial ischemia markers – TnI, CPK-MB, LDH, lactate, as well as myocardial biopsy of the left ventricular apex myocardium before and after donor organ ischemia period.

Myocardial oxygen consumption was calculated according to the formula:

$$\text{LV } O_2 \text{ cons.} = \frac{([O_2]_a) - ([O_2]_{cs}) \times \text{CAF}}{\text{LVmass}},$$

$$\text{ml-}O_2\text{/min/100 g},$$

where, $[O_2]_a$ is arterial blood oxygen content, $[O_2]_{cs}$ is coronary sinus oxygen content, CAF is coronary arterial flow, LV mass is left ventricular myocardium mass.

Blood oxygen content was calculated using the formula:

$$O_2 = \frac{\%O_2\ Sat \times [Hb] \times O_2\ capacity\ of\ Hb\ (1.34\ ml - O_2/g)}{100},$$

$$ml\text{-}O_2/dl.$$

Surgical technique of the experiment

Donor: heart explantation and heart preservation technique

Donor piglets with an average body weight of 33 ± 3.2 kg received premedication and anesthesia according to the technique described above. In all cases, access to the heart was performed through a median sternotomy. After heparin injection in a dose of 3 mg/kg of body weight, a 7 Fr cardioplegic cannula was placed in the aortic root. In the control group after vena cava occlusion, the aorta was clamped and cardioplegia was performed by injecting 2 liters of Bretschneider cardioplegic solution (Custodiol®, Germany, HTK) into the aortic root at 75 mm Hg pressure for the first minute and then at 40 mm Hg for the subsequent 9 minutes. The hearts were then stored in the appropriate solution at 0 to 1 °C. In the experimental group, the hearts were subjected to persufflation according to the technique described in Fischer J. [19]. We used modified HTK (mHTK) solution (with 40 mg/L of hyaluronidase added) to initiate cardioplegia, then we placed an aortic valve blocker that was cut from glove rubber in the form of trefoil and fixed with one knot stitch in the center. Moistened carbogen (95% O_2, 5% CO_2) was introduced into the ascending aorta through a transverse incision or through the brachiocephalic trunk, maintaining pressure in the aortic root at 40–45 mmHg. The heart was placed in a plastic bag filled with mHTK solution and surrounded by ice chips. Drainage tubes were placed in the right and left ventricular cavity, the free ends of which were left in the solution to determine free gas escape. After 3 hours of preservation, we proceeded to graft preparation and implantation to the recipient.

Recipient: donor heart implantation

Piglets weighing 25 ± 1.7 kg underwent median sternotomy. After heparin injection at a dose of 3 mg/kg of body weight, appropriate cannulas were inserted into the right common carotid artery and vena cava. After initiation of cardiopulmonary bypass (CPB), the donor heart was explanted leaving a wide cuff of pulmonary veins; the recipient's body was cooled to 28 °C. Orthotopic implantation of the donor heart was performed using bicaval technique by consecutive anastomosis of the

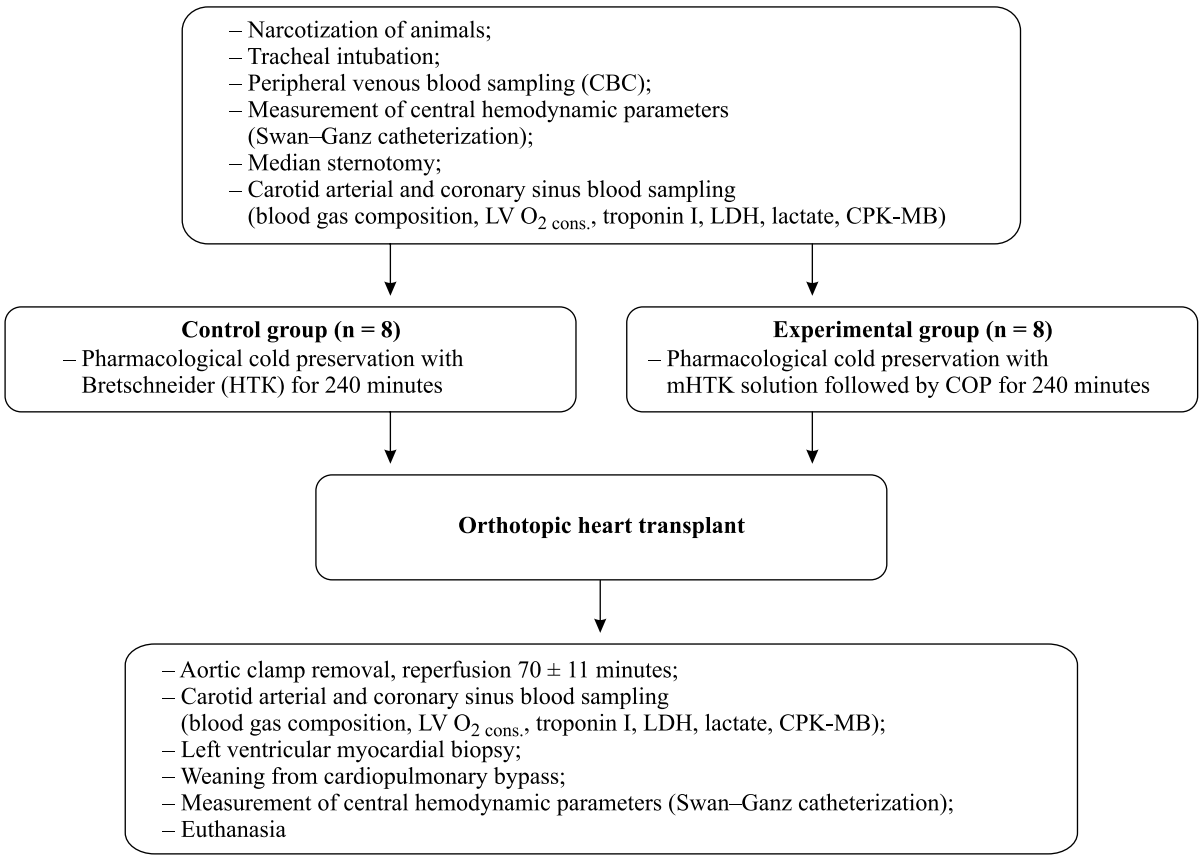

Fig. 1. Study protocol

left atrium, pulmonary trunk, aorta, inferior and superior vena cava. For the purpose of immunosuppression, all recipients received pulse methylprednisolone therapy (Metipred®Orion, Portugal) at a 1,500 mg dose before aortic clamp removal and reperfusion. In the persufflation group, donor heart implantation was performed without cessation of coronary gas supply up to formation of aortic anastomosis. Cardiac reperfusion was started with a 10-minute warming of the heart with oxygenated modified Krebs–Henseleit solution, containing only 50 μmol/L calcium and 15 μmol/L adenosine at 50 mm Hg pressure to remove gas bubbles from the capillary bed. During the first minutes of reperfusion, blood samples were taken from the arterial line of the heart-lung machine and the coronary sinus in order to calculate myocardial oxygen consumption and determine the level of myocardial ischemia markers. Thirty minutes after the clamp was removed from the aorta, myocardial biopsy of the left ventricular apex was performed. The recipient's body was gradually warmed and weaned from CPB. After 2 hours of observation, euthanasia was performed by injecting 100 mL of 4% potassium chloride solution under general combined anesthesia (4–7 mg/kg propofol, 0.006–0.008 mg/kg fentanyl, and 2–4 vol% sevoflurane inhalation).

Myocardial specimens for histological examination were excised from the apical region of the left ventricle, fixed in 10% formalin solution on phosphate buffer (pH 7.4) and embedded in paraffin. 5 μm thick sections were prepared on a Microm HM 550 microtome and stained with hematoxylin and eosin according to the van Gieson method with a combined dyeing of elastic fibers with orsein; a Schiff test was also performed. Histology and morphometric studies were performed using a software-microscope complex that included a light microscope (Carl Zeiss), an AxioCam MRc digital video camera, and a Pentium 4 computer.

Statistical processing was performed using Statistica 10.0 software (StatSoft Inc., USA). The normality of distribution was tested using the Shapiro–Wilk test, followed by estimation of equality of variance using Levene's test. If there was normal distribution in the experimental groups and there was intergroup equality of variance, further processing was performed using parametric statistics – Student's t-test. Nonparametric statistical methods were used for distributions other than normal. Differences between the groups were considered significant at $p < 0.05$.

RESULTS

A total of 16 OHTx surgeries were performed during the study. Donor heart ischemia time in the experimental and control groups was 248 ± 12 and 242 ± 10 minutes ($p > 0.05$), respectively; implantation time did not differ significantly between the groups – on average 47 ± 6 and 39 ± 7 minutes ($p > 0.05$), respectively. In all experiments, reperfusion time was 60 ± 8 minutes, after which infusion of cardiotonic drugs (dopamine 10 μg/kg/min, adrenaline 0.1 μg/kg/min) was started for all recipients and gradually weaned from CPB. Changes in cardiac output (CO) were assessed at three points: point 1 – immediately after weaning from CPB; point 2 – 60 minutes after independent graft functioning; point 3 – 120 minutes after independent graft functioning (Table 1). In both groups, there was a significant decrease in CO after weaning from CPB in comparison with the baseline values. However, the differences between the groups were statistically non-significant ($p > 0.05$).

In the persufflation group, recovery of cardiac pump function required more active antiarrhythmic and cardiotonic support. In all cases in the COP group, there was stable ventricular fibrillation, and restoration of correct rhythm required multiple (up to 10) attempts at defibrillation. In contrast, all animals in the control group showed spontaneous recovery of coordinated heartbeats.

Changes in LDH, TnI and lactate concentrations in blood flowing out of coronary sinus are presented in Table 2. Comparative analysis revealed no statistically significant difference between the groups ($p > 0.05$).

Myocardial oxygen consumption was significantly lower in the persufflation group after reperfusion ($p = 0.011$). However, when compared between groups, oxygen consumption did not differ ($p > 0.05$).

The histological picture of myocardial parenchyma and stroma of control and experimental animals was generally similar. When subjected to H&E stain, muscle fibers of normal size, sarcoplasm of muscle segments were uniformly and moderately accepting of eosin (Fig. 2). Transverse striation was clearly detected in the longitudinally cut fibers, and areas of mild myofibril contracture were noted in some places. The nuclei of muscle fibers were mostly medium-sized, oval bacilliform

Table 1

Changes in cardiac output (L/min)

Group	Baseline	After CPB	In 60'	In 120'
Control (n = 8)	3.36 [3.36; 3.97]	2.35* [2.14; 2.71]	3.03 [2.96; 3.34]	2.99 [4.85; 3.17]
Experimental (n = 8)	3.72 [3.15; 4.28]	2.15*$^{\#}$ [2.01; 2.42]	2.95$^{\#}$ [2.25; 3.12]	2.48$^{\#}$ [2.04; 2.92]

Note: *, $p < 0.05$ versus baseline; $^{\#}$, $p > 0.05$ versus control group.

or elongated, uniformly stained dark blue with clumps of chromatin with distinct nuclei.

In the control group, the epicardial stroma was moderately and irregularly edematous. The arteries and veins were with wide oval lumen; around part of vessels, there was slight perivascular edema, single lymphocytes in capillaries. In the experimental group, unlike the control group, the marginal standing of lymphocytes in capillaries was diffuse; there was slight perinuclear edema in some cardiomyocytes; vessel dilatation with round contour preservation. In both groups, endothelial cells were evenly distributed, flatly arranged and retained their integrity (Fig. 3).

DISCUSSION

Deficit of ischemia time is one of the main factors limiting the geography of donor bases and, accordingly, the possibilities of donor potential. Currently, donor heart ischemia time in clinical practice is limited to 3–5 hours in the case of cold preservation [20]. Unfortunately, the current cold preservation method involves replenishment of all deficits during ischemia except one – oxygen. Donor organ perfusion systems, as well as hyperbaric oxygenation devices, are not widely used in clinical practice due to its cumbersomeness and high cost of its consumable components [20–22]. In contrast to continuous perfusion methods with oxygen-containing preservation solution or blood, COP technique that was discovered more than a century ago does not require complex perfusion equipment. Persufflation is a combination of primary cardiac arrest by cold method followed by continuous antegrade delivery of gaseous oxygen into coronary arteries.

Despite the results of numerous studies demonstrating high efficacy of COP as a method of long-term (14 hours) graft conditioning, so far it has had no impact on the attitude of clinicians towards the idea of intentional filling of the coronary bed with gas mixture [9–11, 23]. The first full-fledged studies on the efficacy and safety of coronary persufflation were performed in 1959; Sabiston D. et al. in a series of experiments on anterograde COP (A-COP) with humidified carbogen gas (95% O_2, 5% CO_2) showed that canine hearts can continue to contract for 5 hours (2.5–8 hours) ex vivo while maintaining normothermia [24]. In the next series of experiments, the authors performed A-COP in situ for 25–30 minutes, after which they were able to restore normal coronary blood flow. At the same time, a majority of animals had complete restoration of the hemodynamic function of the heart. The main conclusions of this study were that: the heart is able to use gaseous oxygen by direct persufflation; successful restoration of myocardial contractility is possible after A-COP and coronary reperfusion with blood.

Later in 1960, Talbert J. et al. introduced the concept of retrograde COP (R-COP) [25]. At that time, retrograde perfusion of oxygenated blood through coronary sinus was actively used to maintain cardiac rhythm and protect the heart against short-term ischemia during open aortic valve interventions [26, 27]. In their studies, the authors injected carbogen through the coronary sinus, which allowed to maintain heart beats for an average of 3.5 hours, and in case of additional cannulation of the anterior heart veins, up to 5.5 hours. Later in 1966, Camishion R. et al. published an article describing the results of R-COP in aortic valve interventions [23]. The term ‘persufflation’ officially replaced ‘gaseous oxygen perfusion’ in 1971 [14] after which the interest in research on persufflation declined significantly.

In the 90’s, persufflation became a subject of research again. In 1998 for instance, Kuhn-Regnier F. et al. published a study on the use of A-COP as a method of heart conditioning before orthotopic allotransplantati-

Table 2

Changes in the levels of biochemical markers in blood flowing out of the coronary sinus

Indicator \ Group	Control (n = 8)		Experimental (n = 8)	
	before OHTx	after OHTx	before OHTx	after OHTx
LDH, U/L	429.85 [355.8; 546.3]	693.60* [491.25; 778.87]	442.05 [329.4; 555.8]	773.25*$^{\#}$ [654.35; 948.67]
TnI, pg/mL	5.15 [2.35; 8.17]	48.45* [26.53; 73.75]	4.85 [2.55; 7.37]	67.10*$^{\#}$ [27.78; 104.8]
Lactate, mmol/L	1.45 [1.12; 2.02]	9.55* [8.53; 10.25]	1.30 [1.05; 2.12]	9.55*$^{\#}$ [8.53; 10.25]
CPK-MB, U/L	204.00 [166.5; 324]	326.15* [225.5; 453.25]	168.00 [118; 324]	376.15*$^{\#}$ [225.5; 535.75]

Note: LDH, lactate dehydrogenase; CPK-MB, creatine phosphokinase; OHTx, orthotopic heart transplantation; *, $p < 0.05$ versus pre-heart transplant level; $^{\#}$, $p > 0.05$ versus control group.

Table 3

Myocardial oxygen consumption (ml-O_2/min/100 g)

Group	Baseline	After reperfusion	P
Control (n = 8)	9.15 [7.17; 11.9]	8.2 [7.35; 9.35]	0.31
Experimental (n = 8)	10.6 [8.18; 15.42]	7.7$^{\#}$ [6.75; 10.12]	0.011

Note: $^{\#}$, $p > 0.05$ versus control group.

on in the experiment [28], a similar study was published by Fischer J. et al. [29]. The average graft ischemia time in these studies was 14.5 hours. The authors described significant advantages in recovery of cardiac output, coronary blood flow, left ventricular pressure and myocardial relaxation after a long period of A-COP compared to an isolated cold preservation group [30].

Given pilot study results, it remains unclear why COP has not received widespread support. Perhaps, the "barrier" of direct and intentional introduction of air mixture into the vascular bed, formed by the general perceptions of clinicians about the danger of embolism, still makes them skeptical about the safety of COP. The technical conduct of persufflation, given the need for continuous gas delivery to the aortic root throughout the cardiac implantation stage, has no significant impact on the course of operation. Moreover, we did not obtain evidence indicating a negative effect of COP on cardiac pump function restoration compared with the control group; the study of myocardial ischemia markers revealed a significant increase in LDH, TnI, CPK-MB, and lactate concentrations in blood flowing from the coronary heart both in the persufflation group and in the control group. Morphological studies also showed no significant ischemic myocardial damage compared with the control group. The integrity of the endothelial lining of vessels and their patency were preserved. Besides, it is necessary to take into account the possible effect of the absence of any crossmatch selection on the results, with the development of acute graft rejection.

CONCLUSION

In the course of the experiment, the technical feasibility and safety of direct intracoronary oxygen persufflation at the stage of donor heart conditioning ex vivo was proved. At the same time, experiments found

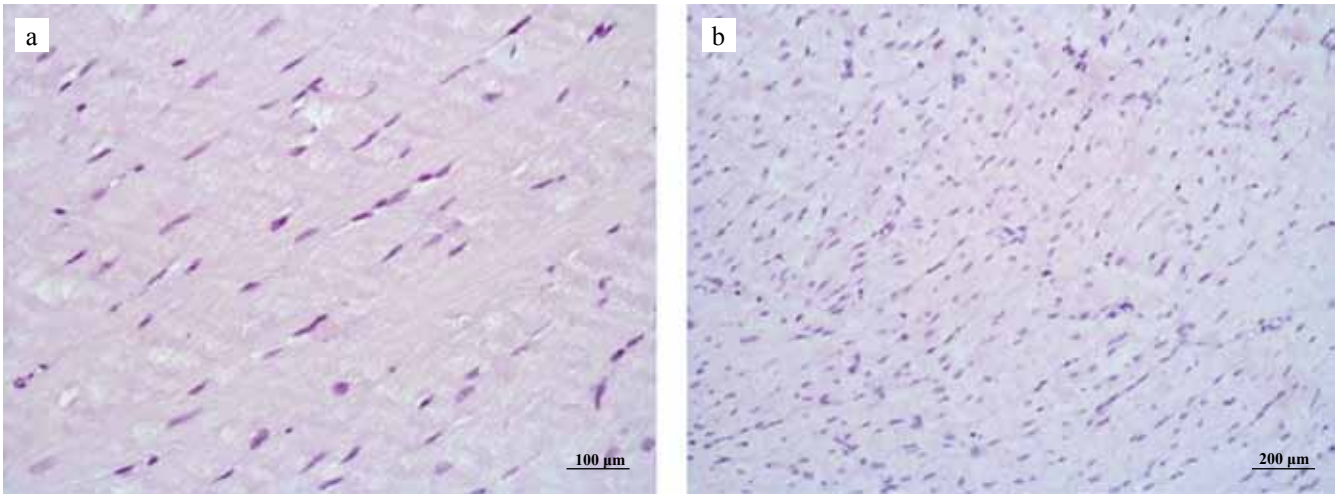

Fig. 2. Left ventricular myocardium with preserved muscle fiber diameters and mild contractures; a, control group. H&E staining. 400× magnification; b, experimental group. H&E staining. 200× magnification

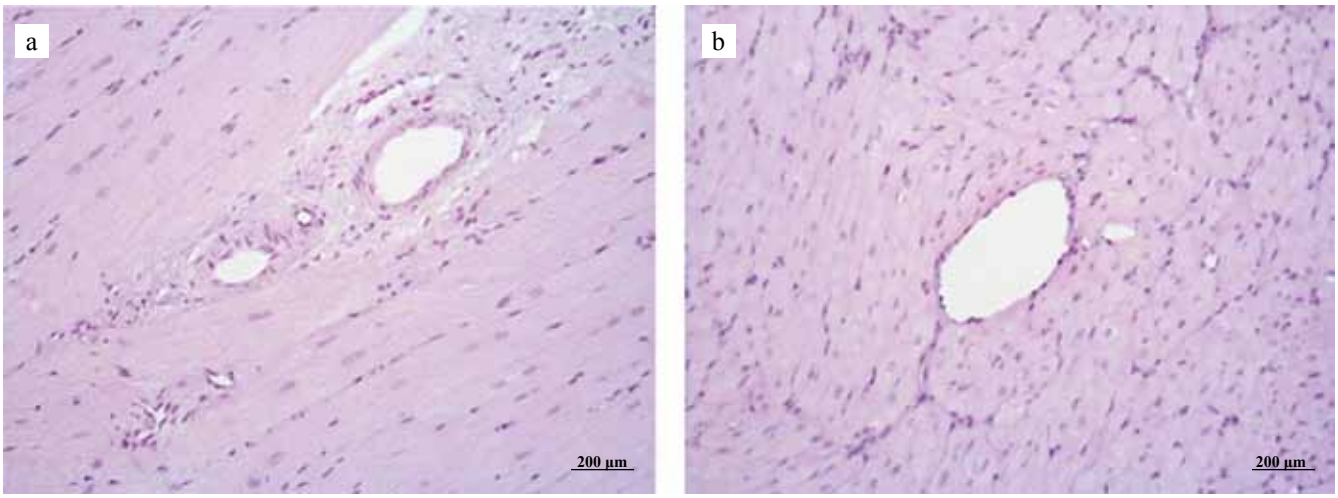

Fig. 3. Left ventricular intramyocardial vessels. Preserved endothelial lining; a, control group; b, experimental group. H&E staining. 200× magnification

no significant advantages of coronary persufflation over the standard protocol of cold preservation of donor heart by Bretschneider cardioplegic solution. The absence of significant differences in functional, biochemical and structural integrity of the graft between the groups may be due to the short organ preservation period and short observation period, which requires more extensive and long-term observations.

The authors declare no conflict of interest.

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USING A NEW SELECTIVE ANTEGRADE CEREBRAL PERFUSION TECHNIQUE FOR ASCENDING AORTA AND AORTIC ARCH REPAIR

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Dissecting aortic aneurysm is one of the most dangerous diseases of the aorta, often leading to severe complications or death. Currently, due to the increased level of diagnosis and the speed of care for patients with aortic diseases, there is now a need to improve approaches to the treatment of this condition. This paper presents the outcomes of a technique developed at our center, Shumakov National Medical Research Center of Transplantology and Artificial Organs, for selective antegrade cerebral perfusion (SACP) when performing prosthetic replacement of the aortic arch under circulatory arrest. Surgical treatment is performed on an emergency basis. During these surgeries, we focused on preventing neurological complications. Analysis of the efficacy and safety of our SACP technique shows that we obtained positive outcomes. In the analysis of 10 cases of aortic arch replacement, there was no evidence indicating the presence of any neurological complication. This technique allows for more adequate monitoring of perfusion during reconstructive interventions on the ascending aorta and aortic arch than the classical perfusion technique.

Keywords: ascending aorta and aortic arch repair, dissecting aortic aneurysm, selective antegrade cerebral perfusion, circulatory arrest, ischemic brain injury.

Aortic arch surgery remains one of the most complex areas of cardiac surgery. Dissecting aneurysm is the main condition requiring surgical intervention on the ascending aorta and aortic arch (Fig. 1).

Aortic dissection (AD) is the formation of a tear in the inner elastic layer of the aorta with subsequent blood inflow into the degeneratively altered middle layer, formation of intramural hematoma and spread to the inner and outer layers with the formation of an additional intravascular channel (false lumen) (Fig. 2). Dissection occurs more commonly in the distal (antegrade) direction, less common in the proximal (retrograde) direction [1, 2].

Dissecting aortic aneurysm is a medical emergency (requiring surgical correction as soon as possible) that, even with optimal treatment, can quickly lead to death. If AD goes through all the three layers of the aorta, a complete tear through all the layers occurs, resulting in massive bleeding. Aortic rupture carries a mortality rate of 80%, and half of patients die in the prehospital phase [3, 6–10].

Arterial hypertension is one of the main causes of dissecting aneurysms. Most patients with AD have this disease. Connective tissue dysplasia and congenital diseases are often combined with Marfan syndrome, Ehlers–Danloh syndrome, congenital bicuspid aortic valve, aortic coarctation, Turner syndrome, giant cell aortitis and recurrent polychondritis and other diseases, which are also important factors in dissecting aneurysms.

Pregnancy is also a risk factor for AD; 50% of AD afflicting women younger than 40 years are pregnancy associated, the highest incidence being in the third trimester [2–7]. Aortitis, blunt chest trauma, aortic atherosclerosis combined with hypertension and preeclampsia in pregnant women are also accompanied by aortic dissection and even aortic aneurysm rupture as a complication [2, 4, 5].

Dissecting aortic aneurysms have been reported following surgical procedures where counterpulsation devices are inserted into the aorta, or the aorta or its major branches are cannulated. It is believed that iatrogenic dissecting aortic aneurysm is a rare complication. Unlike the spontaneous one, iatrogenic dissection is observed in older age groups and is more often accompanied by atherosclerosis. Trauma rarely leads to dissecting aneurysm [4–10].

The classification of dissecting aortic aneurysms is based on the localization of the proximal intima tear and the extent of dissection.

The AD classification proposed by Michael Ellis DeBakey (1965) (Fig. 3) provides an anatomical description of the dissection variants. They divided dissection according to the site of onset and extent of dissection:

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Type I – onset of dissection in the ascending aorta, further extends to the aortic arch and often distally beyond;

Type II – confined to the ascending aorta;

Type III – onset in the descending aorta, but spreads distally, rarely spreading proximally.

According to the Stanford classification of AD (1970), all cases of aortic tears can be divided into two groups – A and B, depending on which section of the aorta is involved.

Group A involves dissection in the ascending aorta and/or aortic arch and possibly the descending aorta. It includes DeBakey types I, II, and III. Retrograde dissection (occurring in the descending aorta or aortic arch, but spreading to the ascending aorta) is also possible.

Group B involves dissection in the descending aorta (distal to the origin of the left subclavian artery), without involvement of the ascending aorta or aortic arch. It includes DeBakey type III without retrograde continuation into the ascending aorta [2, 6, 7, 10].

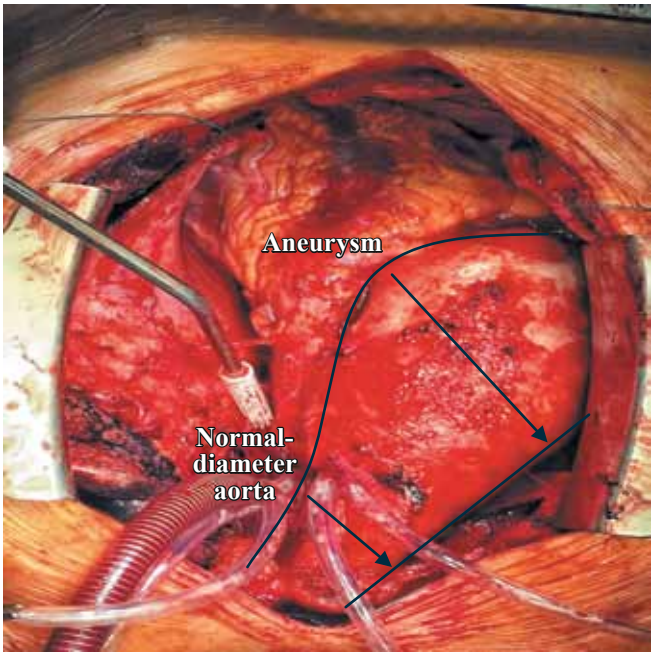

Fig. 1. Aortic aneurysm

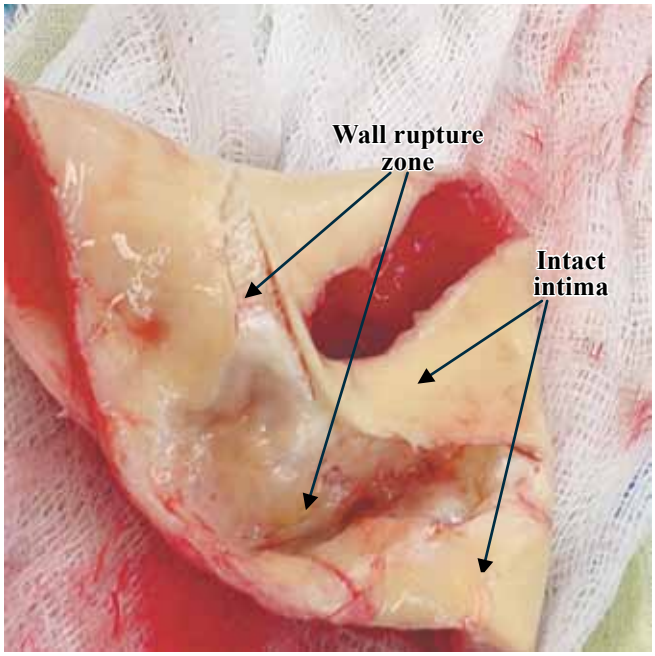

Fig. 2. Excised section of the dissected ascending aorta

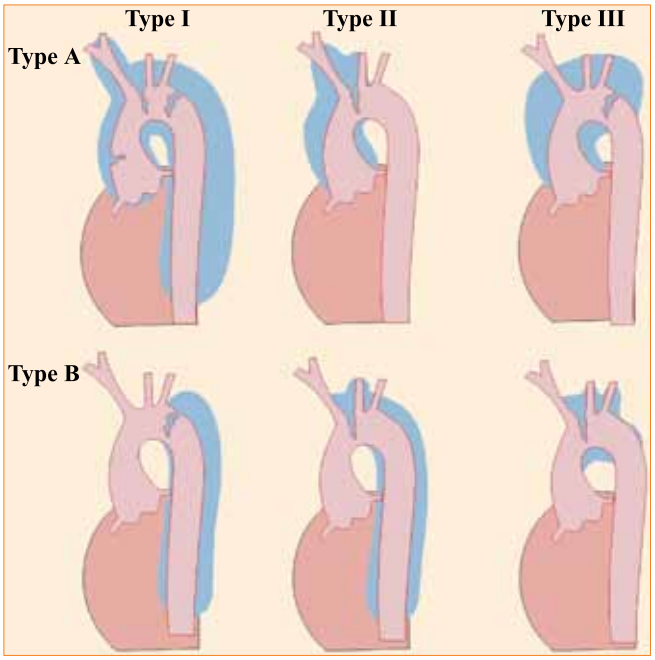

Fig. 3. Classification of aortic dissections by prevalence: DeBakey (1965) and Stanford (1970)

Currently, two main mechanisms of dissecting aneurysm formation are considered: aortic intima rupture or stretching and intramural hematoma development. This mechanism is the most common and is more frequently mentioned in literature. Aortic endothelial layer rupture usually occurs due to hypertension and/or dilatation of the vessel. The aortic layers separate due to the impact of pulse wave. The most common intima rupture forms in the ascending aorta, immediately above its sinuses. In 60% of cases, the tear is localized on the anterior surface of the ascending aorta, distal to the left subclavian artery in 30%, and within the aortic arch in 10% [6, 10].

Much more complicated is the second AD mechanism. The rupture of vasa vasorum and, as a consequence, formation of intramural hematoma, spreading within the middle layer of the aortic wall, leads to intima rupture. This mechanism occurs in no more than 10% of cases.

Despite advances and improvements in surgical and perfusion techniques over the past 30 years, mortality, morbidity and, in particular, incidence of cerebral complications, remain higher than for procedures performed

on the more proximal aorta. Traditional approaches in the prevention of cerebral injury have focused on the use of the following concepts during aortic arch replacement: deep hypothermic circulatory arrest (10–15 °C), retrograde cerebral perfusion through jugular veins under moderate hypothermic circulatory arrest (25–30 °C), and selective antegrade cerebral perfusion under moderate hypothermic circulatory arrest (25–30 °C) – a technique that most world clinics now follow [11].

WAYS TO PROTECT THE BRAIN

Deep hypothermic circulatory arrest (DHCA) confers advantages to the surgeon, allowing work to be done in a bloodless and cannula-free operating field. However, there are a number of significant disadvantages. First, the cooling and warming process increases cardiopulmonary bypass (CPB) time significantly and leads to associated postoperative organ dysfunction. Severe coagulopathy and increased associated risks of uncontrolled spontaneous bleeding also develop [11]. Secondly, during the warming period, there is a probability of ischemic cerebral reperfusion injury [12], disruption of the normal mechanisms of cerebral circulation regulation and formation of excessive cerebral temperature gradients [13], which aggravate other sources of cerebral injury. Thirdly, the formation of anastomoses by open technique during DHCA increases the risk of material and air emboli entering both cerebral and distal vessels. The time available for performing complex aortic arch reconstruction during DHCA is limited, as prolonged periods of arrest are associated with a proportional increase in the incidence of cerebral and target organ injury [14]. It should be noted that minimal impairment of brain function can occur with DHCA lasting even 20 minutes [10]. This potentially indicates that any period of DHCA can cause adverse neurological outcomes. Such time limitation can lead to technically imperfect aortic arch reconstruction, which in turn can lead to early or late complications.

In an attempt to ensure adequate cerebral blood flow and thus prolong the "safe" DHCA period, many centers have introduced adjuvant cerebral perfusion techniques. Some centers dealing with aortic surgery in large volumes have successfully adapted the use of retrograde cerebral perfusion (RCP) in combination with DHCA [13]. However, there are currently published experimental and clinical studies showing a limited supply of nutrients to the brain in the RCP and suggesting excessive retrograde perfusion pressure and associated cerebral edema that aggravate cerebral injury [4, 5].

In contrast to RCP, SACP is more widely used and provides adequate cerebral blood flow [8]. The methods fall into 2 main groups: 1) separate cannulation of individual vessels, and 2) use of cannulation sites of aortic arch branches such as the axillary, innominate or carotid artery. Nevertheless, both methods have their disadvantages. Direct cannulation of branches through the open arch can lead to atheromatous and air embolism. In addition, since there are periods of circulatory arrest before and after perfusion cannula insertion, deep hypothermia is necessary. On the other hand, catheterization of aortic arch branches in the periphery of the operative field (e.g., axillary artery) avoids many of these disadvantages, but has its own. Aortic arch reconstruction period is performed with a single inflow and depends on collaterals potentially providing flow transfer to the contralateral hemisphere. This creates the possibility of ipsilateral hypoperfusion and/or contralateral hyperperfusion, depending on whether the flow is excessive or too lean.

It is important to note that both antegrade and retrograde cerebral perfusions do not provide perfusion of other vital organs; they rely entirely on deep hypothermia to protect such organs as kidneys, liver and spinal cord. It is sometimes claimed that SACP provides perfusion of distal organs through upper and lower body collaterals [13]. However, it is questionable whether such a collateral flow provides real nutrient flow under open distal anastomosis, when most of the flow inevitably goes through the path of least resistance – directly into CPB pump suctions. It is important to note that vital organ malperfusion can easily go unnoticed because the consequences are not as obvious as acute stroke. But despite this, its effects can be just as dangerous, manifesting as coagulopathy, gastrointestinal bleeding, sepsis and multiple organ failure [5].

The aim of this study is to analyze our own experience with the original SACP technique in ascending aorta and aortic arch repair to solve the problems of improving the protocol of perfusion support for surgical reconstruction of the aortic arch and the procedure as a whole.

MATERIALS AND METHODS

The retrospective study included 10 patients who underwent reconstructive operations on the ascending aorta and aortic arch between 2019 and 2021 under CPB, circulatory arrest, and SACP using the technique we developed, patent No. RU2734136C1.

The currently known SACP techniques do not allow control of the volume perfusion rate in isolation in the arteries of each cerebral hemisphere; they also do not allow accurate assessment of perfusion pressure in the brachiocephalic trunk (BCA) and the left common carotid artery (LCCA). After cessation of circulatory arrest, pressure control in the BCA and LCCA is possible only with the help of an occluder on the cerebral perfusion line or in one common line, which is bifurcated on the operating table using an adapter – a tee. The available techniques do not allow separate control and correction of the patient's brain and body temperature.

In addition, the known methods are complicated because they require Doppler ultrasound of the BCA, LCCA, and left axillary artery, as well as transcranial Doppler ultrasound of the middle cerebral artery.

In this regard, the bilateral SACP technique developed at Shumakov National Medical Research Center of Transplantology and Artificial Organs was used.

This method allows cerebral perfusion by a bilateral selective cerebral perfusion system combined with a physiological unit such that it becomes possible to perform separate supply of oxygenated blood through the BCA and LCCA with a given volumetric rate and temperature under pressure control in each line (Fig. 4).

This system includes: cardiotomy reservoir (1), first pump (2), oxygenator (3) whose exit is connected to an arterial trunk port (6), LCCA port (17), BCA port (18), second pump (11), characterized by the fact that it further includes a circulation reservoir (7), heat exchanger (9), third pump (12), first (5), second (13) and third (14) pressure sensors, six 4, 8, 10, 15, 16, 19 branch tees, wherein the outlet of the oxygenator (3) is connected in series with the arterial trunk port (6) through the first branch tee (4) and the first pressure sensor (5), one port of the first branch tee (4) is connected to the first port of the second branch tee (8), which consistently connects the inlets of the circulation reservoir (7) and heat exchanger (9); the outlet of the heat exchanger (9) through the third tee (10) is connected to the LCCA and BCA ports by individual lines, one of which contains the second pump (11) and the second pressure sensor (13), and the other contains the third pump (12) and the third pressure sensor (14), and the output of the circulation reservoir (7) is connected through the fourth (15), fifth (16) and sixth (20) tees to the LCCA and BCA lines.

The advantage of the bilateral SACP system used lies with the possibility of isolated control and correction of the volumetric perfusion rate and perfusion pressure in both cerebral hemispheres, as well as independent thermoregulation of the circulatory circuits of the patient's brain and body.

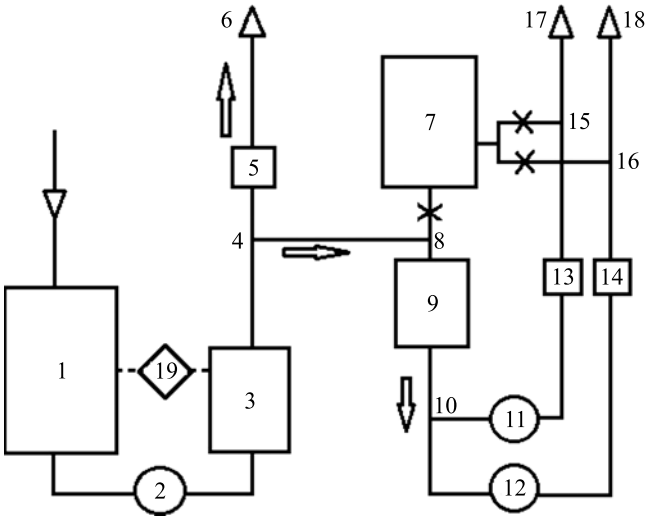

Fig. 4. System for bilateral selective antegrade cerebral perfusion during reconstructive surgery on the aortic arch under cardiopulmonary bypass

The medical and technical outcome is to prevent and reduce neurological complications, decrease the frequency of multiple organ failure, provide for early patient activation and is aimed at reducing hospital mortality during operations on the aortic arch and its branches, performed under hypothermic circulatory arrest with cold cardioplegia due to prolonged hypothermia at the stage of patient warming, control of perfusion rate and pressure in the arteries of each cerebral hemisphere.

The stage of intervention on the ascending aorta consisted of supracoronary ascending aortic replacement, aortic root replacement with a valvulated tube with implantation of coronary artery orifices into it (according to Hugh Bentall and Antony De Bono procedure). The stage of intervention on the aortic arch consisted of full or partial aortic arch replacement with a multibranched prosthesis. In the presence of descending thoracic aortic dissection, Hans Borst's elephant trunk procedure was used to stop the false channel function.

RESULTS

This article also presents analysis of the case histories of 10 patients admitted with De Bakey type I and type II aortic dissection.

There were 5 male and 5 female patients. The average age of the patients was 51.2 ± 14.5 years.

Of the entire group, 10% (1 patient) underwent reoperation under CPB (aortic root reimplantation by David procedure with Gelweave-30 synthetic prosthesis, mitral valve annuloplasty with MedInj-34 support ring, tricuspid valve annuloplasty with MedInj-30 support ring and exoprosthetic repair of the ascending aorta and aortic arch with Gelweave-32 synthetic prosthesis under CPB, circulatory arrest and SACP); the remaining 90% were operated on initially.

Among all patients, 40% had chest pains on admission. At the same time, 70% of the patients complained of shortness of breath of varying intensity. Pathological aortic murmurs during initial examination were detected in 4 patients out of 10. Three patients were hospitalized in severe conditions and underwent emergency surgery for life-threatening indicators. It is worth noting that there were preoperative rhythm disturbances in 30% of patients, where 20% initially had atrial fibrillation and 10% had supraventricular extrasystole. No rhythm disturbances were noted in the remaining patients. The mean heart rate (HR) was ~72 ± 16.37 bpm. Preoperative examination of patients included echo and chest MSCT with intravenous contrast. The mean diameter of the aortic valve fibrous ring (FR) was 2.36 ± 0.13 cm; that of the sinotubular junction (STJ) was 3.91 ± 0.59 cm; mean diameters of the ascending aorta and aortic arch were 5.63 ± 1.05 and 3.8 ± 0.45 cm, respectively. Analysis of left ventricular (LV) volume fractions showed:

mean end-diastolic volume (EDV) of 133.9 ± 57.3 mL; mean end-systolic volume (ESV) of 58.2 ± 28.69 mL; mean Stroke volume (SV) of 75.8 ± 28.35 mL; and mean ejection fraction (EF) of 55.7 ± 7.44%. In 70% of cases, 50 to 100 mL of fluid was found in the pericardial cavity according to echo. Patients with bicuspid aortic valve (AV) were found in 20% of the examined patients, whereas the majority were patients with tricuspid. The mean peak gradient was 10.07 ± 3.66 mm Hg. Most patients (70%) had grade 2 aortic regurgitation; in the mitral valve (MV), 80% had grade 1 regurgitation. There were two cases of grade 2 regurgitation, and one of them had previously undergone MV repair with the use of an annuloplasty ring. Phenomena of group 1 pulmonary arterial hypertension were observed in 30% of patients.

According to MSCT data, aortic dissection on CT scan was detected in all patients; 80% had DeBakey type I AD, and 20% had type II AD.

The extent of surgical intervention in the selected patients was distributed as follows:

– 40% had supracoronary ascending aortic and aortic arch replacement (Fig. 5, a);
– 20% had supracoronary ascending aortic and hemiarch replacement (Fig. 5, b);
– 10% had ascending aorta replacement with a valvulated tube with implantation of coronary artery orifices according to Kouchoukos procedure + aortic arch replacement;
– 30% had aortic arch replacement with multibranched prosthesis by lowering the synthetic prosthesis into the descending thoracic aorta according to Hans Borst's elephant trunk procedure (Fig. 5, c).

The above surgeries lasted for an average of 326.5 ± 62.10 minutes. The aortic clamping time averaged 94.2 ± 45.34 minutes. Selective cerebral perfusion time was 49.4 ± 40.78 minutes on average. All surgeries were also performed under moderate hypothermia, with an average temperature of 25.9 ± 2.06 °C.

All patients required cardiotonic support after surgery, predominantly with dopamine at an average dose of 3.6 μg/kg/min, and dobutamine at an average dose of 2.25 μg/kg/min. In two cases, adrenaline was administered at 80 and 10 ng/kg/min, respectively. It should be noted that both cases of adrenaline use were in patients who underwent emergency surgery for life-threatening indicators.

Perfusion rate and flow (Table 1) reflect the quality of myocardial protection. Coronary perfusion was performed by selective antegrade in all 10 cases. Custodiol solution (2 L) was used for cardioplegia in 30% of cases, and Calafiore blood-based potassium cardioplegic solution was used in the remaining cases.

Table 1 indicates that the mean values of coronary artery perfusion were within acceptable range in all episodes of cardioplegia. In particular, the absence of high resistance in the coronary arteries and optimal flow rates through both cannulas indicate the adequacy of myocardial protection during the above-mentioned operations. Cardioplegia was performed with a solution based on 15% KCL + $MgSO_4$ + lidocaine at a perfusion rate of 150–200 mL/min for 2.5 minutes.

Due to the new SACP technique, cerebral perfusion parameters were also determined during circulatory arrest and during anastomosis formation (Table 2).

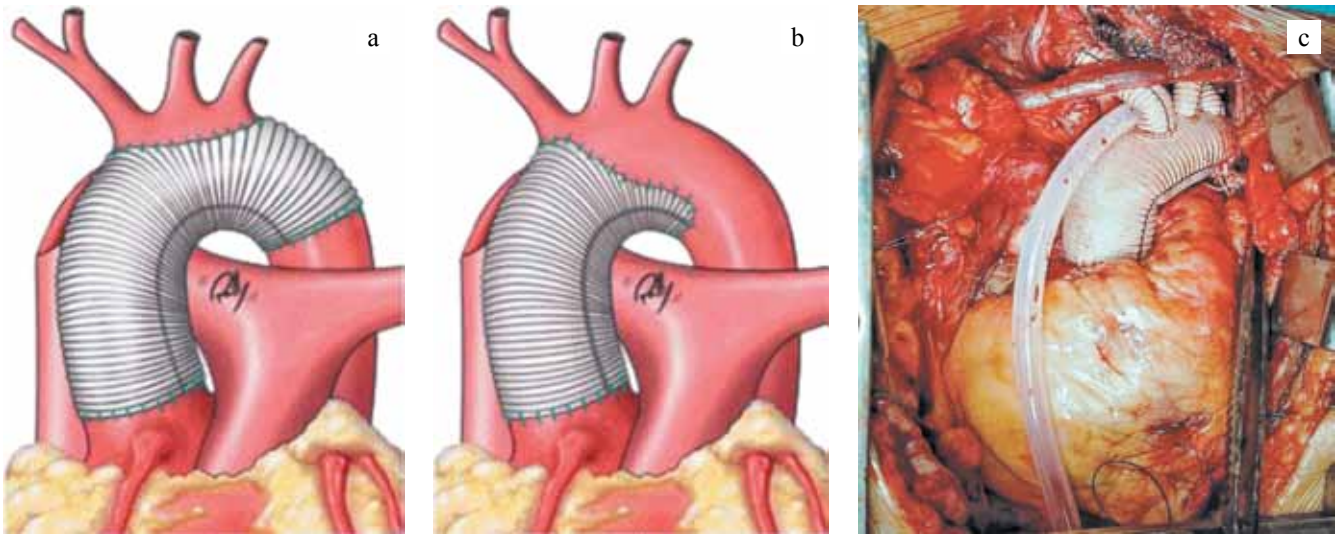

Fig. 5. Types of surgical interventions. a, prosthetic replacement of the ascending aorta and aortic arch (https://www.researchgate.net/figure/230826243_fig1_Figure-3-Supracommissural-replacement-of-the-ascending-aorta-b-hemiarch-replacement-c); b, supracoronary ascending aortic and hemiarch replacement (https://www.researchgate.net/figure/230826243_fig1_Figure-3-Supracommissural-replacement-of-the-ascending-aorta-b-hemiarch-replacement-c); c, supracoronary ascending aortic and aortic arch replacement with a multi-branch prosthesis by lowering the synthetic prosthesis into the descending thoracic aorta according to Hans Borst's elephant trunk procedure

Table 2 shows perfusion rate and resistance values in the BCA and LCCA. These characteristics allowed to exert control throughout the cerebral perfusion, as well as to regulate the flow in each hemisphere separately (using NONIN Sen Smart cerebral oximeter), which in turn increased the possibility of preventing hypoperfusion and hyperperfusion episodes.

Optimal perfusion rates and resistance values in BCA and LCCA throughout the period from cannulation to anastomosis indicate a well-performed brain perfusion. Cerebral oximetry ($SctO_2$), performed with a NONIN Sen Smart oximeter, averaged 68 ± 4.3% on the right and 66 ± 6.2% on the left, which also reflects the effectiveness of the applied perfusion technique. The mean values of cranial oximetry during the entire operation were within 63–72%.

Laboratory values presented in Tables 3 and 4 in turn suggest that there were no significant metabolic disorders during the operations.

Online monitoring of arterial and venous blood gas composition was performed during the entire period of CPB (CPI-500 device was used). Blood parameters were calibrated and monitored every 30 minutes from the beginning of CPB using laboratory diagnostics. This allowed us to maintain optimal concentrations of the main blood gas and ionic composition parameters. These indicators varied throughout the CPB period but remained within normal values due to regular monitoring and correction of metabolic disorders.

Table 1

Indicators of the selective antegrade cardioplegic perfusion conducted

Cardioplegia cannula	Flow (mL/min)	Resistance (mm Hg)
LCA, avr ± st. dev.	185 ± 14.4	100.1 ± 8.4
RCA, avr ± st. dev.	156.2 ± 21.6	106 ± 10.6

Note: LCA, left coronary artery; RCA, right coronary artery.

Table 2

Characteristics of the antegrade cerebral perfusion performed using the new technique

Perfusion cannula	Flow (mL/min)	Resistance (mm Hg)
BCA, avr ± st. dev.	165 ± 23.6	67.1 ± 16.7
LCCA, avr ± st. dev.	182 ± 12.4	70.8 ± 18.1

CONCLUSION

Based on the outcomes of our SACP technique, the clinical efficacy of this procedure has been confirmed. It allows full-scale monitoring of perfusion volume, peripheral resistance of the vascular bed in each hemisphere, controlling the level of oxygenation and independently thermoregulating the circulatory circuits of the patient's brain and body.

The presented results also show that the method is safe and potentially contributes to early activation, reduction in incidence of neurological complications, incidence of multiple organ failure and hospital mortality during interventions on the aortic arch and its branches performed under hypothermic circulatory arrest with cold cardioplegia.

Table 3

Acid-base balance parameters of arterial blood, reflecting metabolic changes during cardiopulmonary bypass (mean values are presented)

Time	HCO_3, mmol/l	pCO_2, mmHg	pO_2, mmHg	sO_2, %	Hb, g/l	Lac, mmol/l	K^+, mmol/l	Na^+, mmol/l	pH	A(BE), mmol/l	S(BE), mmol/l
5 min	24.9	32.1	263.5	99.8	91.1	1.33	4.55	117.5	7.45	0.5	0.62
30 min	23.35	28.37	224.7	99.1	91.12	1.5	4.61	132.12	7.49	–0.6	–0.42
60 min	21.31	26.53	223.96	98.8	88	2.76	5.23	131.66	7.47	–2.98	–3.58
90 min	20.3	30.22	198.8	98.6	82.08	3.9	4.02	134.8	7.40	–5.06	–4.8
120 min	21.75	31.25	210.75	99.1	86.6	3.6	3.9	136	7.42	–3.25	–3.07

Table 4

Acid-base balance parameters of venous blood, reflecting metabolic changes during cardiopulmonary bypass (mean values are presented)

Time	HCO_3, mmol/l	pCO_2, mmHg	pO_2, mmHg	sO_2, %	Hb, g/l	Lac, mmol/l	K^+, mmol/l	Na^+, mmol/l	pH	A(BE), mmol/l	S(BE), mmol/l
5 min	24.72	33.38	82.45	86.55	91.62	1.42	4.56	133.75	7.47	0.48	0.6
30 min	23.11	29.22	64.23	85.85	60.42	1.75	4.56	132.25	7.47	–1.33	–1.02
60 min	22.23	32	28.55	78.43	87.33	3.06	5.63	131.83	7.42	–2.46	–2.21
90 min	20.94	35.48	33.9	79.92	81.44	4.12	3.98	134.2	7.36	–4.32	–3.84
120 min	22.2	37.6	37.975	78.9	86.27	3.05	3.92	135.75	7.38	–2.6	–2.27

Against the background of short CPB and circulatory arrest time, sufficient perfusion, moderate hypothermia, and maintenance of target hematocrit level, the surgical outcomes look predictable and logical: a short stay in the intensive care unit, in the absence of permanent neurological deficit. No cardiac and renal complications, hospital and 30-day mortality.

Careful surgical hemostasis against the background of high-quality anesthesia allowed to minimize the number of resternotomy surgeries for bleeding to 1 case out of 10.

According to MSCT data, the radicality of the performed reconstructions on the ascending aorta and aortic arch in the postoperative period is beyond doubt. In the first days after surgery, sinus rhythm was restored in 100% of the patients with an average frequency of 79 ± 12.37 bpm. According to echo, performed on day 2 after the operation: mean diameter of the aortic valve FR did not change – 2.36 ± 0.13 cm; mean diameter of the STJ was 3.52 ± 0.15 cm; mean diameter of the ascending aorta and aortic arch was 3.18 ± 0.19 cm and 3.8 ± 0.45 cm, respectively.

The results of the immediate outcome assessment also did not reveal significant brain ischemia. This complication was detected in one patient, caused by the initial presence of cerebral ischemia zones in the patient.

The authors declare no conflict of interest.

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DECELLULARIZED HOMOGRAFT FOR AORTIC VALVE REPLACEMENT TWO YEARS AFTER LUNG TRANSPLANTATION

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Cardiac valvular surgery in patients after lung transplantation is a challenging procedure, reports are scarce. We report a 29-year-old patient who underwent concomitant mitral valve reconstruction and implantation of a decellularized aortic homograft two years after bilateral lung transplantation.

Keywords: decellularized homograft, cardiac surgery after lung transplantation.

INTRODUCTION

If lung transplanted patients develop heart valve disease, young age and the need for immunosuppression, regular transbronchial biopsies, and redo transplantation may influence operative strategy. While valvular reconstruction should be pursued whenever possible, the balance between the risk of bleeding in case of a mechanical substitute and future structural valve deterioration (SVD) in case of biological prostheses, should guide valvular prosthetic choice in case of replacement [1]. Recently, decellularized homografts have showed promising hemodynamics and durability and may represent an alternative to conventional biologic prosthesis [2].

We report the implantation of a decellularized aortic root allograft in a lung-transplanted patient who underwent concomitant mitral valve repair.

CASE DESCRIPTION

A 29-year-old female with severe pulmonary hypertension due to capillary hemangiomatosis was put on the waiting list for lung transplantation in December, 2017. The pre-transplant transthoracic echocardiography (TTE) showed trivial aortic and mitral insufficiency without morphological disturbances of the valve apparatus, severe impaired right ventricular function, mild tricuspid regurgitation and severe pulmonary hypertension.

In May, 2018 she developed right heart failure, was put on ECMO and transplanted 8 days later with ECMO removed by the end of surgery. The patient was discharged two months later.

In January, 2020 she was admitted at our institution due to new-onset dyspnea (NYHA III). Acute cellular and humoral rejection as well as infection were excluded. However, the TTE (Fig. 1, a and c) showed severe mitral regurgitation due to fibrosis without annulus dilatation (Carpentier IIIa), moderate-to-severe aortic regurgitation, a slightly decreased left ventricular ejection fraction (LVEF, 50%) with a left ventricular end diastolic diameter (LVEDD) of 55 mm. The tricuspid aortic valve showed fibrotic changes and central regurgitation due to failing leaflet coaptation, with low mean pressure gradient of 3 mmHg. The aortic annulus measured 22 mm. The left heart catheterization showed normal coronary arteries, and the right heart catheterization showed a normal pulmonary artery pressure (27/14/18 mmHg), a wedge pressure of 18 mmHg, a pulmonary vascular resistance of 22 Dynes, and a cardiac index of 2.24 (l/min)/BSA.

After median sternotomy, cannulation of the proximal aortic arch and both venae cavae was performed. The mitral valve morphology included isolated fibrotic restriction, more pronounced in the anterior leaflet (Fig. 1, b). Trans-septal mitral valve repair with anterior leaflet augmentation using untreated autologous pericardium, and an annuloplasty with a 26 mm ring was performed. The aortic valve leaflets appeared retracted (Fig. 1, d), showed restricted movement, and thus were not amenable to repair. The decellularized homograft with an annulus diameter of 21 mm was implanted orthotopically with a running suture [2]. Operation, bypass and cross clamp time amounted to 312 min, 225 min and 162 min, respectively. We used cold blood cardioplegia and mild hypothermia (32 °C). Intraoperative volume balance amounted to 3800 ml. Postoperatively the patient was extubated 5.5 hours after arrival at the intensive care unit and was transferred to the normal ward the next day requiring only 1 L oxygen supply over the nasal catheter.

Immunosuppressive therapy was replaced by a continuous intravenous infusion of 200 mg hydrocortisone

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24 hours before operation. On the first postoperative day, the immunosuppressive therapy with prednisolone (10 mg), tacrolimus (target level 8–10 ng/dL) and mycophenolate mofetil (750 mg twice a day) was reinitiated. Antibiotic therapy included meropenem and flucloxacillin for 7 days.

On the 11th postoperative day, she was discharged to the rehabilitation clinic after uncomplicated postope-

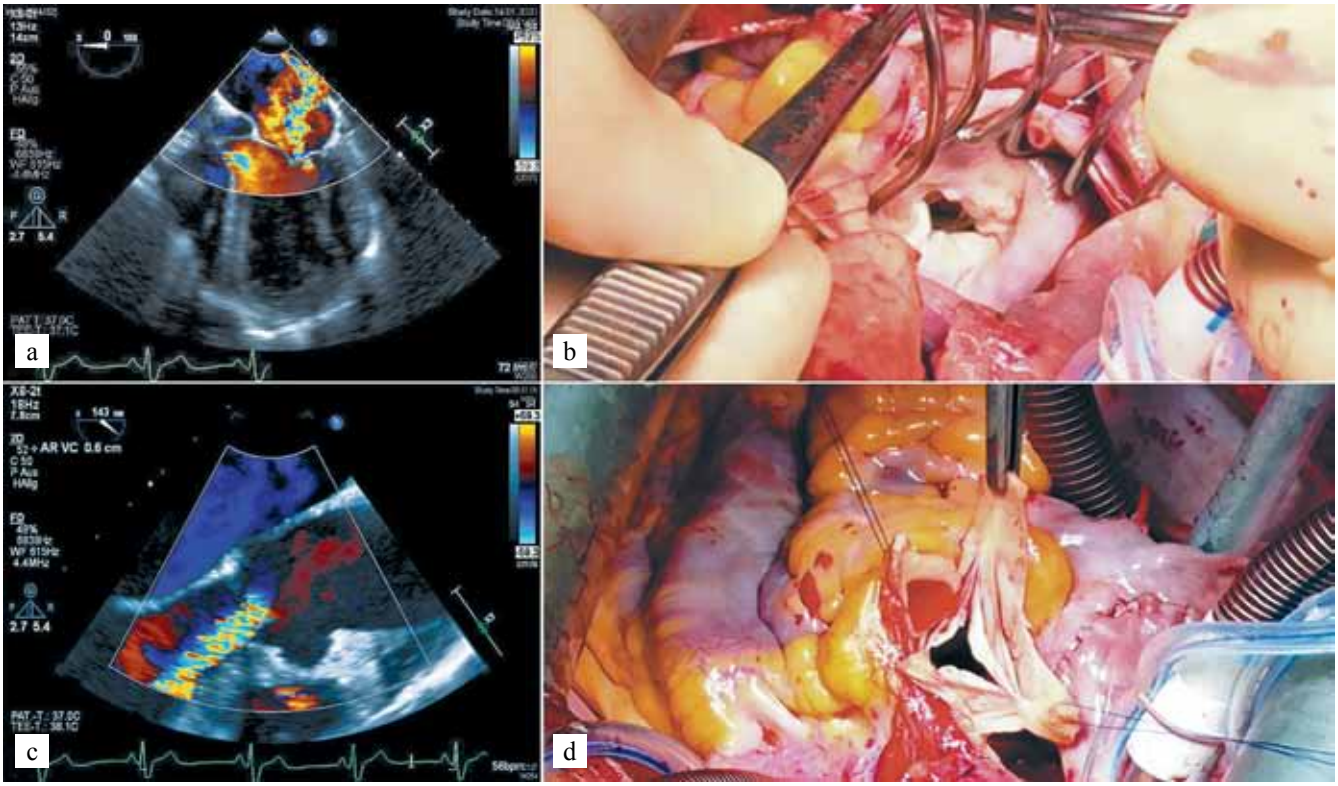

Fig. 1. Transesophageal echocardiography and intraoperative findings of the mitral (a, b) and aortic (c, d) valves

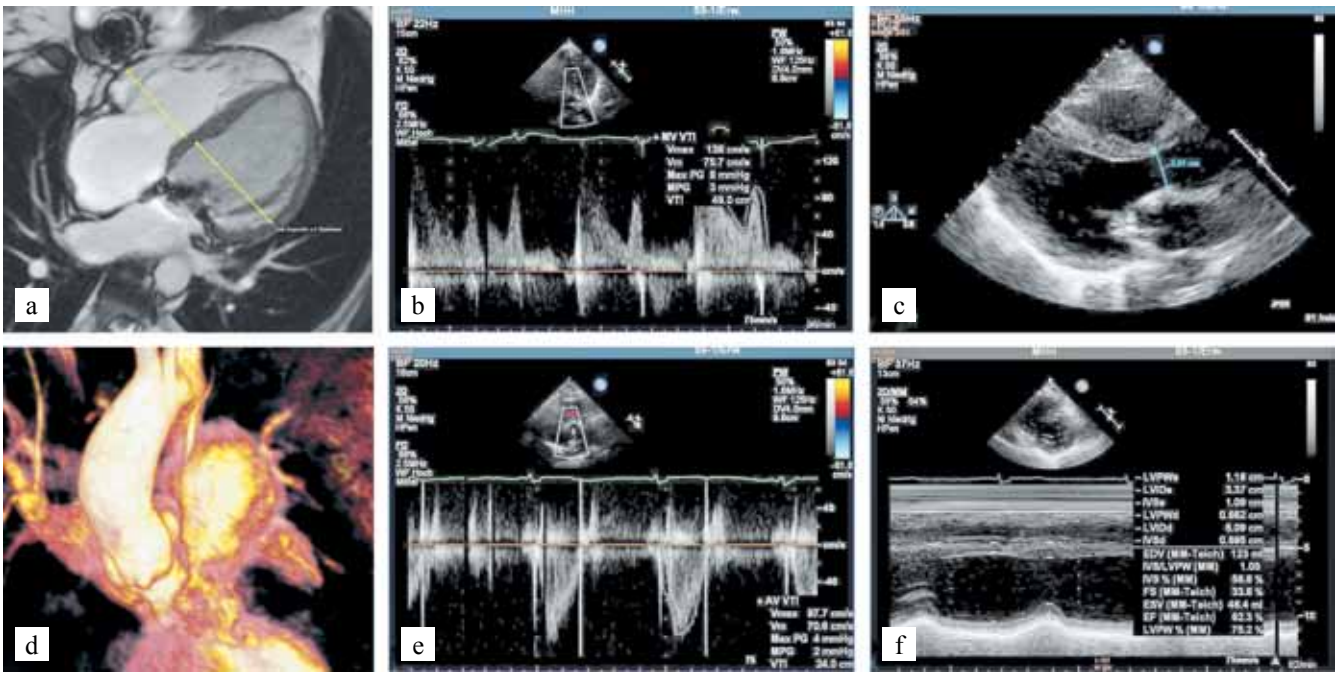

Fig. 2. Postoperative findings: a – four chamber view showing normal heart dimensions; b – pw-Doppler signal in reconstructed mitral valve; c – B-mode systolic longitudinal axis showing wide LVOT and good mitral coaptation; d – contrast enhanced MRI-angiography of LVOT; e – pw-Doppler signal in aortic annulus; f – M-Mode dimensions of the LV

rative course. TTE performed at discharge did not show any mitral or aortic valvular regurgitation. Moreover, the left ventricular function amounted to 60% with LVEDD of 43 mm, the aortic valve area to 2 cm^2, the mean pressure gradient across the mitral valve to 3 mmHg by an opening area of 3 cm^2. The lung function tests showed FEV_1 of 1.23 L (41% predicted), VC_{max} of 1.81 (52% predicted), FEV_1/VC_{max} of 68% (81%).

At 3 months follow up the TTE showed excellent aortic valve function, mild mitral insufficiency and the mean pressure gradient across the mitral valve was 3 mmHg. The MRI revealed normal heart dimensions, the aortic valve had a maximum gradient of 6 mmHg by a maximal flow speed of 122 cm/s and a mild regurgitation (Fig. 2).

COMMENT

Experience in lung-transplanted patients undergoing cardiac valve surgery is scarce [3–5]. According to our recently published experience, concomitant lung transplant and cardiac valvular surgery yielded poor results [6]. Contrarily, cardiac surgery after lung transplantation showed better early and long-term results. In the present case report, we had to face several challenges off, and we discussed several strategies for planning the operation. TAVI for aortic valve replacement was not possible, because the aortic valve was not stenotic. Singular mitral valve clipping may have temporarily reduced the symptoms, but may not have provided acceptable long-term durability [7]. Therefore, we planned reconstruction of both valves. The mitral valve morphology allowed a safe repair with augmentation of the anterior leaflet using autologous pericardium, achieving sufficient coaptation length, and an annuloplasty using a 26 mm ring. The preoperative echocardiography showed fibrosis and restricted movement of the aortic leaflets, as well as a small aortic annulus (22 mm). According to the current guidelines for aortic valve surgery [1], our 29-year old patient should have received a mechanical prosthesis. However, we considered the need for lung biopsies and the concrete risk of developing chronic lung allograft dysfunction and requiring re-transplantation in the future an important contraindication for implanting a mechanical prosthesis. However, conventional biologic prostheses undergo rapid SVD in young patients [1]. Furthermore, the small aortic annulus of our patient would only have allowed the implantation of a relatively small prosthesis, either mechanical or biologic. Instead, we have recently reported that decellularized homografts, showed significant reduction in the re-operation rate in comparison to conventional biologic prosthesis in children and young adults [8]. Moreover, aortic valve replacement with a homograft is best suited for patients with a small aortic annulus to provide a better effective orifice area and lower gradients after surgery.

Axel Haverich holds shares in corlife oHG. Igor Tudorache is medical consultant for corlife oHG. The other authors of this manuscript have no conflicts of interests.

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TWO-STAGE RECONSTRUCTIVE PLASTIC SURGERY FOR GIANT OLEOGRANULOMA OF THE EXTERNAL GENITALIA IN A KIDNEY RECIPIENT

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The paper presents the clinical case of successful treatment of giant oleogranuloma of the external genitalia in a kidney recipient. The relevance of the problem, which has not diminished over time due to identification of new cases, is presented. Stages involving pathological tissue removal and reconstructive plastic surgical intervention to restore the anatomical form and functionality of the penis are described and illustrated in detail. The authors conclude that reconstructive plastic intervention for penile oleogranuloma can be effectively performed in a patient with end-stage chronic renal disease treated via renal transplant and who is receiving triple immunosuppressive therapy.

Keywords: kidney transplantation, oleogranuloma of the penis, reconstructive plastic surgery.

INTRODUCTION

The number of kidney transplantations (KTx) performed in Russia every year is on the rise. Despite improvements in dialysis methods of treatment, the duration and quality of life in patients with a transplanted kidney remain high [1]. The increasing number of observations of patients with a transplanted kidney has led to identification of paradoxical complications arising in the post-transplant period and not previously described in medical literature. We present a clinical case of successful two-stage surgical treatment of a giant oleogranuloma of the external genitalia in a transplant kidney recipient.

Oleogranuloma of the male external genitalia remains an urgent problem of modern urology [2, 6]. Oleogranuloma of the external genitalia is a pathological condition that develops after injection of oily-like substances into the penile skin. It is represented by a cascade of inflammatory and trophic changes in the skin-facial cover [6]. The patient takes these actions in order to provoke an inflammatory response to the introduction of a foreign agent, development of lymphostasis and subsequent increase in penis size. Introduction of oily substances (Vaseline oil, paraffin, baby cream, various ointments) is done at home, fortunately, as a rule, in compliance with aseptic rules. Over time, the pathological process manifests as inflammatory and trophic changes in the skin and penile fascia (due to subcutaneous injection of the above oily substances) and formation of dense infiltrates on the penile body with subsequent genital scar deformity, edema and pain during erection [3, 7]. Penile oleogranuloma is often complicated by paraphimosis, ulcerous defects and fistulas. Diagnosis of oleogranulomas is usually straightforward and is made on the basis of previous injections of oily substances under the skin, physical and radiological examinations [4].

There are three stages of the disease depending on the extent of the pathological process [5]. In the first stage, the pathological process spreads to one third of the surface of the penile shaft; in the second stage, it spreads to the entire surface; and in the third stage, the process spreads to the scrotal skin, perineum, and suprapubic area.

Conservative treatment, both in the acute and chronic stages of the disease, turns out to be ineffective. Surgical treatment is basic and is aimed at radical removal of granulation tissue and deposits of the injected foreign agent. The efficiency of surgery depends on the degree of severity of the pathological process and the presence of complications. Patients with the third stage of oleogranuloma are considered the most difficult. The main method of treatment in the third stage of the disease is two-stage Reich–Sapozhkov operation. No cases of oleogranuloma in patients on immunosuppressive therapy have been described in the available literature.

The aim of this paper is to present a clinical case of a patient with a transplanted cadaveric kidney, who is on a triple immunosuppressive therapy and underwent two-

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stage reconstructive plastic surgery for a giant oleogranuloma of the penis, scrotum and suprapubic soft tissues.

MATERIALS AND METHODS

Patient G., born on November 23, 1978, was diagnosed in 2010 with chronic glomerulonephritis (not histologically verified) with nephrosclerosis, end-stage chronic kidney disease. Replacement therapy by long-term hemodialysis was initiated. On September 29 of the same year, the patient underwent an allotransplantation of a kidney obtained from a deceased donor to the left iliac region. From anamnesis, it was also established that in 2008 and 2012, the patient had injected a total of about 25 mL of Vaseline ointment into his penile area on his own. From 2017, he started noticing a progressive increase in scrotal and penile volumes due to a dense infiltrate. By 2019, the infiltrative inflammation had spread to the soft tissues of the suprapubic area. It should be noted that since transplantation, the patient has been monitored by a nephrologist at his place of residence, where there was routine correction of immunosuppressive therapy. He had refused the proposed surgical treatment all these years.

On October 9, 2020, the patient was routinely admitted to the urology department of the branch of Shumakov National Medical Research Center of Transplantology and Artificial Organs in Volzhskiy for the purpose of surgical treatment.

At the time of admission, the patient's general condition was satisfactory, he was fully conscious and active. Body temperature was normal. Skin and visible mucous membranes, except for the pathological focus, were of physiological color and there was normal humidity. Breathing was independent, free, hemodynamics was stable. Independent urination was preserved. Bowel and bladder functions were normal. Renal graft function was satisfactory, creatinine level was 115 μmol/L. Immunosuppressive therapy: 4 mg/day methylprednisolone, 10 mg/day tacrolimus divided into two doses and 1080 mg/day mycophenolic acid divided into two doses.

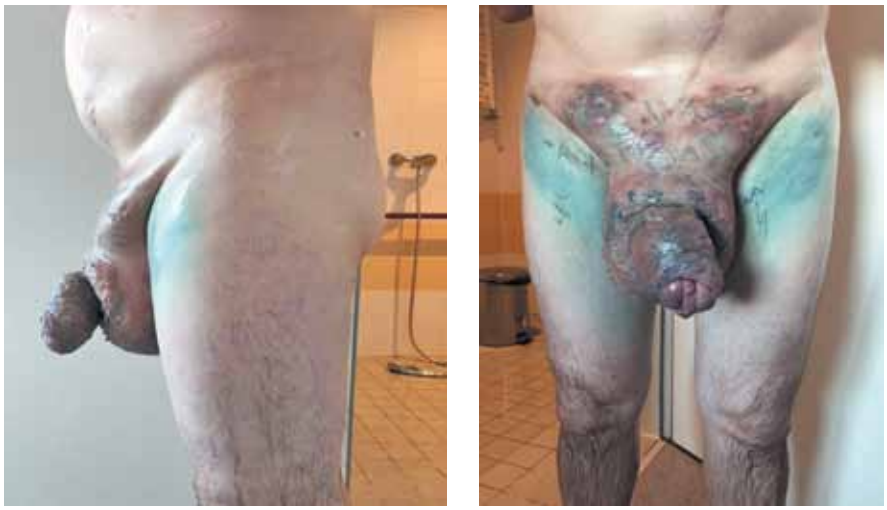

Fig. 1. View of the external genitalia before surgery

Fig. 2. Estimated volume of excised pathological tissues

The external genitalia were shaped like those of a man. The penis and scrotum were sharply enlarged in size. The skin on the genitals was edematous, with a solid infiltrate of dense elastic consistency, spreading to the suprapubic region, hypogastrium, right and left iliac region. It is impossible to open the glans due to the pronounced infiltrative process and scar deformity of the foreskin (Fig. 1).

The patient was comprehensively examined by a therapist, nephrologist, transplant surgeon and an anesthesiologist: no contraindications for surgical treat-

ment were found. Clinical diagnosis: oleogranuloma of the penis, stage 3. Complete nephrological diagnosis: chronic glomerulonephritis with outcome in bilateral nephrosclerosis. Chronic kidney disease C5(T). Cadaveric renal allotransplantation to the left iliac region on September 29, 2010. Triple immunosuppressive therapy.

RESULTS

On November 10, 2020, the 1st stage of surgical treatment was performed under combined anesthesia: dissection of altered tissues of the scrotum, penis and anterior abdominal wall with plasty using local tissues (Figs. 2 and 3).

The operation lasted for 250 minutes. Blood loss volume was 340 mL. Removed pathological tissues

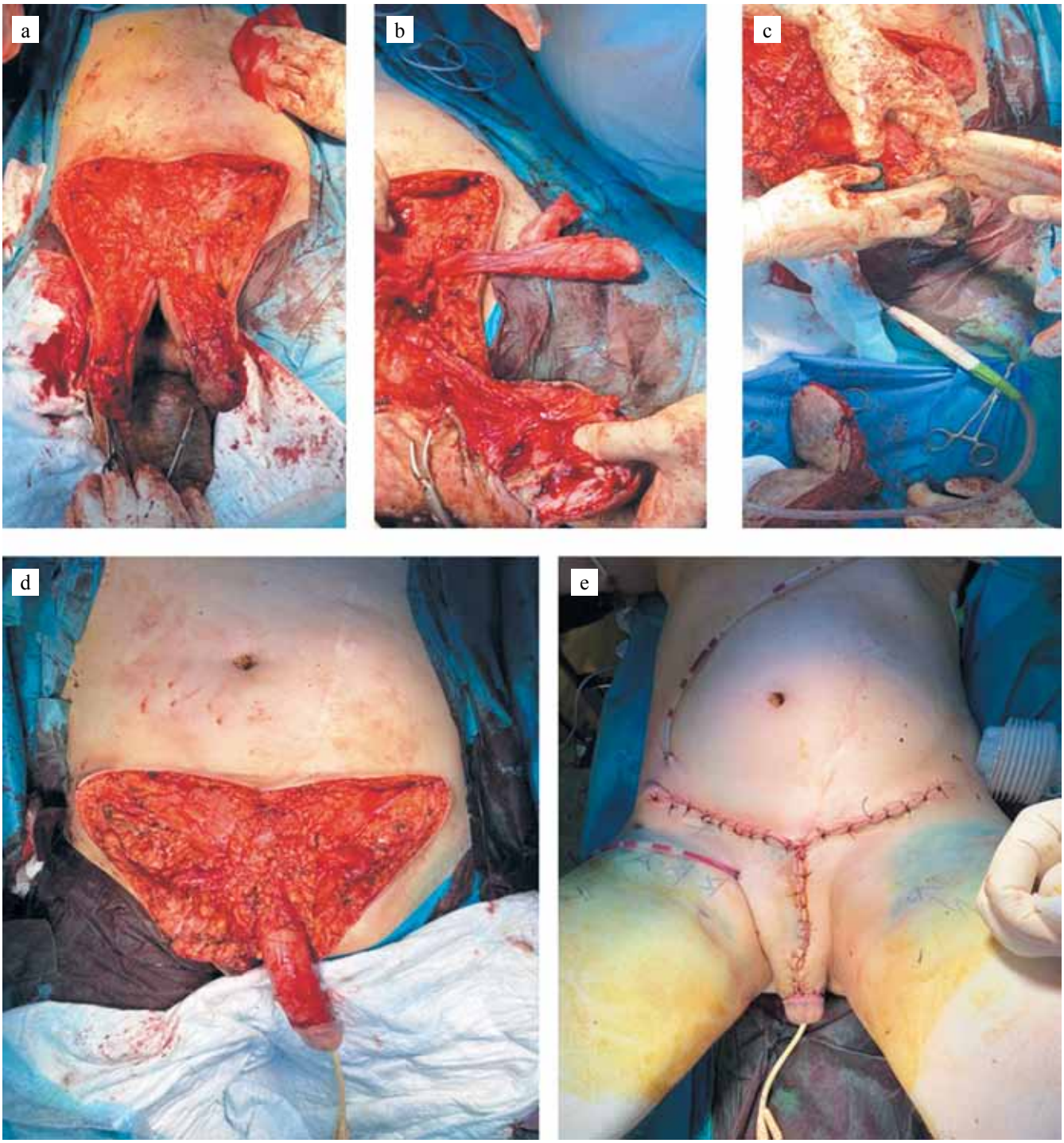

Fig. 3. Surgical intervention: a, wide excision of indurated skin with subcutaneous tissue of the pubic region to the deep fascia; b, testicle isolation and scrotum excision; c, scalping of the penis; d, final view of the surgical wound. The testicles are submerged under the skin of the medial surface of the thighs; d, layered wound closure. The penis is covered by the perineum

weighed 2472 g. Pathohistological examination result: macro specimen was represented by dense fibrous tissue with adipose tissue, with the presence of mixed cellular inflammatory infiltration; there was accumulation of foreign large multinucleated cells. Abundant capillary outgrowths, irregular edema, the epidermis in a state of acanthosis and sharply pronounced papillomatosis. Vacuolization of epidermal upper layers. Stratum corneum consists of parakeratotic cells. Condylomata acuminata.

Renal graft function in the intra- and postoperative periods remained satisfactory; no signs of dysfunction were noted. The patient was discharged on day 10 after surgery, creatinine level was 121 μmol/L at the time of discharge.

Subsequent 7-month outpatient follow-up by physicians demonstrated good repair of the operative area, absence of pathological tissue areas and progression of the local inflammatory process (Fig. 4). Renal graft function was stable.

On June 8, 2021, the patient was readmitted at the urology department for the reconstructive stage of treatment for the penile oleogranuloma. Due to no contraindications for surgical treatment and a stable renal graft function, the second stage of treatment was performed on June 10, 2021: "release" of the penis (Fig. 5).

The operation lasted for 120 minutes; blood loss was 100 mL. The postoperative period was uneventful. The safety drain and Foley catheter were removed on day 2 after surgery. Wound healing mainly occurred by primary tension and partly by secondary tension in the penile root area.

The second stage of surgical treatment also had no adverse effect on renal graft function. The patient was discharged on day 8 after surgery, with 112 μmol/L creatinine level.

The 11-month patient follow-up demonstrated a good clinical outcome (Fig. 6), restoration of erectile function and the possibility of resuming his sexual activity.

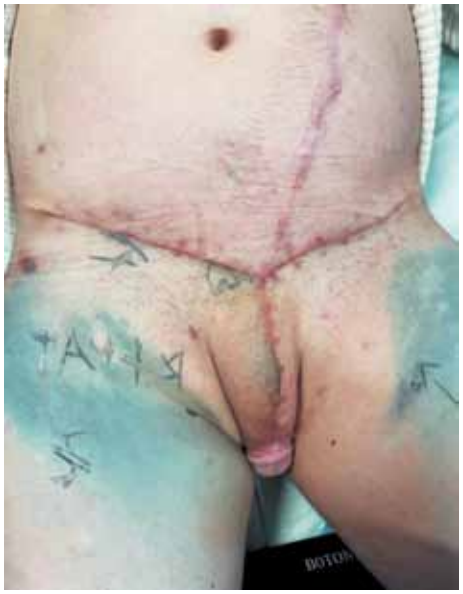

Fig. 4. Patient's condition before the second stage of surgery

CONCLUSION

The clinical case demonstrates the variety of pathological processes that can occur over the course of

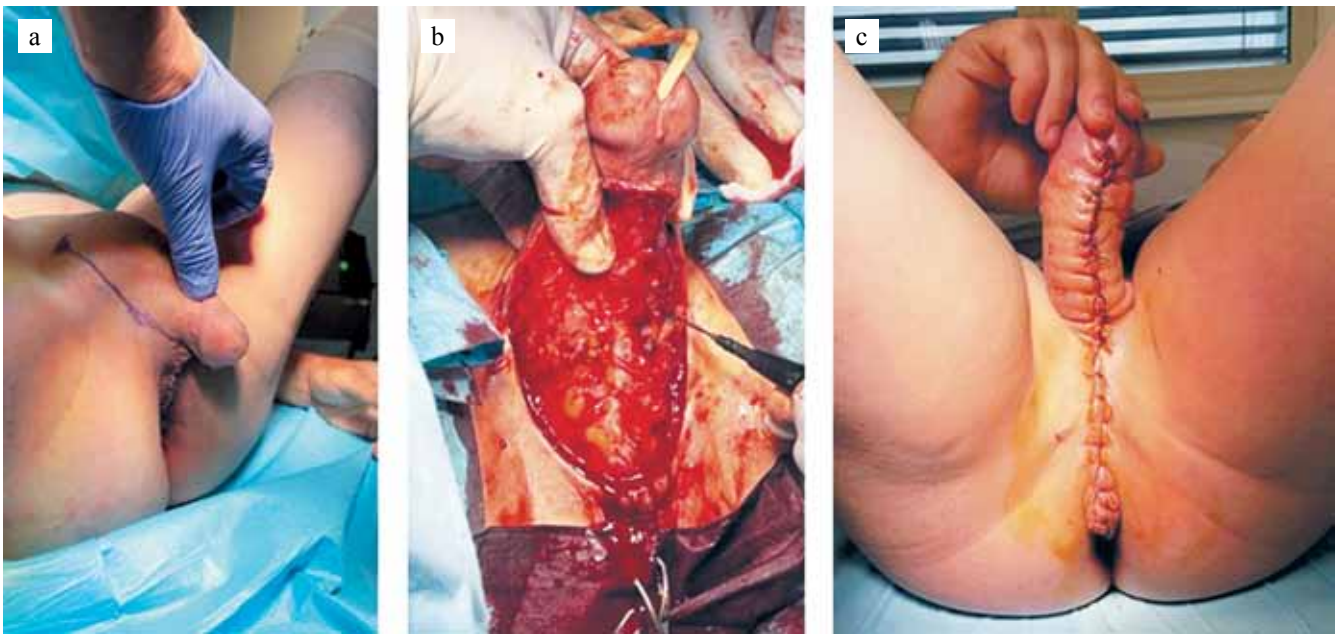

Fig. 5. Reconstructive stage of penile oleogranuloma treatment. a, marking the skin incision line to mobilize the penis; b, U-shaped incision of the perineum; c, final view after penile skin reconstruction on day two after surgery

Fig. 6. Day 40 after surgery

a lifetime in an organ transplant recipient. The severity of the pathological process and the giant size of the oleogranuloma were probably due to the duration of the disease, the patient's reluctance to seek specialized medical care, and the peculiarity of the course while taking immunosuppressive drugs. Oleogranulomas of the external genitalia can occur even in such an exclusive category of patients as kidney recipients, and reconstructive plastic surgery remains the only radical method of treatment.

The authors declare no conflict of interest.

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Abstract

Each article must be accompanied by an abstract. The amount of text for the abstract of the original article should be of no more than 300 words, for a literature review, clinical observation – no more than 200 words. The abstract must fully comply with the content of the work. The abstract should not use abbreviations without prior expansion.

Abstract of ***the original article*** should contain the following sections: ***Objective***, ***Materials and methods***, ***Results***, ***Conclusion***. The abstract should present the most important results of the research.

Do not write: "*A comparative analysis of the sensitivity and specificity was conducted …*"

Should write: "*The sensitivity was ... % and ...%, p = , specificity, respectively ...% and ...%, p =* ".

Keywords

At the end of the abstract keywords must be given. To select the keywords a thesaurus of U.S. National Library of Medicine should be used – Medical Subject Headings (MeSH) at http://www.ncbi.nlm.nih.gov/mesh.

Conflict of interest

The author should inform the editor about the factual or potential conflict of interest have included the information about such conflict into the respective section of an article.

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This information is supposed to be placed before the article text.

Text of article

Original article should include the following sections:

- Introduction
- Materials and methods
- Results
- Discussion
- Conclusion
- References

Review article should include an analysis of the literature with the presentation of modern sources (mainly in the last 5 years).

Clinical observation should be well illustrated (to reflect the essence of the problem) and include discussion with the use of literature data.

References in the text are indicated by number in square brackets: [1], [2, 5], [14–18] and ***in the references section are presented in order of their appearance in the text.*** All values given in the article should be expressed or duplicated in **SI** units.

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The author is solely responsible for the accuracy of the data included in the references section of the article. References to unpublished papers or papers in print works are not allowed.

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Illustrations and drawings should be submitted in electronic format (JPEG or TIFF format with a resolution of at least 300 dpi and no smaller than 6 × 9 cm), in a volume of close to 1 MB. Drawings must include all copyright symbols – arrows, numbers, signs, etc. Figure captions should be submitted in a separate file with the extension *.doc. First, the name is given, then all arithmetic and alphabetical symbols (lettering) are explained.

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