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CLINICAL TRANSPLANTOLOGY

НАЦИОНАЛЬНОМУ МЕДИЦИНСКОМУ ИССЛЕДОВАТЕЛЬСКОМУ ЦЕНТРУ ТРАНСПЛАНТОЛОГИИ И ИСКУССТВЕННЫХ ОРГАНОВ ИМЕНИ АКАДЕМИКА В.И. ШУМАКОВА – 55 ЛЕТ

Глубокоуважаемые коллеги!

2024 год отмечен знаменательным событием для всех нас – 55-летием Национального медицинского исследовательского центра трансплантологии и искусственных органов имени академика В.И. Шумакова. В 1969 году в структуре АМН СССР был создан НИИ трансплантации органов и тканей с целью разработки трансплантологических подходов к лечению наиболее тяжелых заболеваний. Сейчас это специализированное научно-клиническое учреждение, ставшее за более чем полувековой путь ведущим трансплантологическим центром нашей страны, лидером отечественной трансплантологии, обладающим мировой известностью.

Мы отдаем дань памяти и благодарности академику АМН СССР Г.М. Соловьеву – первому директору, возглавлявшему институт в 1969–1974 гг., академику В.И. Шумакову – основателю отечественной трансплантологии, бессменному директору института в период 1974–2008 гг. С 2008 года по настоящее время честь и ответственность возглавлять это славное учреждение и вести его коллектив к новым свершениям на благо нашего отечественного здравоохранения и медицинской науки доверены мне.

В последние годы отечественная трансплантология получила существенное развитие – и как вид высокотехнологичной медицинской помощи, доступный населению нашей страны, и как направление мультидисциплинарных исследований, реализующее научные достижения в клинику, и как область технологических разработок с созданием медицинских изделий и аппаратов, предназначенных для протезирования функции поврежденных органов.

SHUMAKOV NATIONAL MEDICAL RESEARCH CENTER OF TRANSPLANTOLOGY AND ARTIFICIAL ORGANS – 55 YEARS

Dear colleagues,

The year 2024 marks a significant event for all of us – the 55th anniversary of the Shumakov National Medical Research Center of Transplantology and Artificial Organs. In 1969, the Research Institute of Organ and Tissue Transplantation was established under the USSR Academy of Medical Sciences with the goal of developing transplant-based treatments for the most severe illnesses. It is now a highly specialized research and clinical facility that has gained international recognition over the course of more than half a century as Russia's top transplant center and the country's pioneer in transplantology.

We pay tribute to the memory of and express our gratitude to two prominent individuals – Prof. Gleb Solovyov, a fellow of the USSR Academy of Medical Sciences (UAMS), the first director to lead the institute from 1969 to 1974, and Prof Valery Shumakov, also a UAMS fellow and the founding father of organ transplants in Russia, who served as the institute's permanent director from 1974 to 2008. Since 2008, I have been entrusted with the honor and duty of leading this glorious institution and its workforce to new heights for the benefit of our country's national health care and medical science.

In recent years, our national transplantology has developed significantly. It is now a kind of high-tech healthcare that the nation's patients can access, a field of multidisciplinary research that applies scientific discoveries to clinical settings, and a technological frontier with the creation of medical implants and prosthetics intended to replace the functions of damaged body organs.

Яркой демонстрацией значимости и признания успехов, достигнутых в этой области, явилось присуждение коллективу авторов (С.В. Готье, М.Ш. Хубутия, М.Г. Минина) Государственной премии Российской Федерации в области науки и технологий за 2023 год за разработку, научное обоснование и реализацию в практике отечественного здравоохранения трансплантации жизненно важных органов для излечивания тяжелых заболеваний у взрослых и детей.

Трансплантология не стоит на месте. Мы активно разрабатываем и совершенствуем методики сохранения и восстановления донорских органов, системы вспомогательного кровообращения для детей и взрослых, методы поддержания активного долголетия реципиентов солидных органов и многое другое.

Не будет преувеличением сказать, что клиническая трансплантология сегодня – это еще один эффективный путь сбережения нации. Пожалуй, это главная цель и главный результат наших многолетних усилий. У Центра трансплантологии имени академика В.И. Шумакова есть достижения, которыми можно гордиться, есть задачи, которые еще предстоит решать, есть высокопрофессиональный коллектив, способный и умеющий работать.

В этот юбилейный год – 55-летия НМИЦ ТИО им. акад. В.И. Шумакова – особенно хочется пожелать коллективу, всем сотрудникам Центра, действующим и будущим, а также всему нашему трансплантологическому сообществу дальнейших успехов и новых достижений на благо жизни и здоровья нашего народа.

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

A team of authors (Sergey Gautier, Mogeli Khubutia, and Marina Minina) was awarded the State Prize of the Russian Federation for science and technology for 2023 for the development, scientific justification, and implementation in national organ transplant care for the treatment of severe diseases in adults and children. This was a striking demonstration of the significance and recognition of the successes achieved in this field.

The field of transplantology is dynamic. We are actively developing and improving methods of preserving and restoring donor organs, assisted circulation systems for children and adults, methods of prolonging the active lifetime of solid organ recipients, and much more.

It is no exaggeration to say that clinical transplantology today is another effective way of saving the nation. Perhaps, this is the primary objective and the main outcome of our many years of efforts. The Shumakov National Medical Research Center of Transplantology and Artificial Organs has achievements that we can be proud of, but there are still challenges to be addressed and we have a highly skilled team capable and able to work.

On this anniversary year, which marks the 55th anniversary of Shumakov National Medical Research Center of Transplantology and Artificial Organs, I would especially like to wish the team, all past, present, and future employees of the Center, as well as our entire transplant community more success and new accomplishments for the benefit of life and health of our people.

Sincerely,

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

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ORGAN DONATION AND TRANSPLANTATION IN THE RUSSIAN FEDERATION IN 2023

16th Report from the Registry of the Russian Transplant Society

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Objective: to study current trends and advancements in organ donation and transplantation in the Russian Federation based on data from the year 2023. **Materials and methods.** Heads of organ transplant centers were surveyed through questionnaires. The Russian Ministry of Health's information accounting system was used for data control. A comparative analysis of data collected over years from various federal subjects of the Russian Federation and transplant centers was conducted. **Results.** Based on data retrieved from the National Registry in 2023, there were 50 kidney, 34 liver and 22 heart transplant programs existing in the Russian Federation as of the year 2023. Organ donation activity in 2023 was 6.3 per million population (p.m.p.), with a 77.2% multi-organ procurement rate and an average of 2.9 organs procured from one effective donor. In 2023, 3,057 organ transplants were performed in the Russian Federation, which included 1,817 kidney, 829 liver and 388 heart transplants. Same year, the number of transplant surgeries performed in the Russian Federation increased by 19.6% compared to 2022. Organ donation activity in Moscow was 29.1 p.m.p. The city of Moscow and Moscow Oblast alone had a total of 12 transplant centers, which accounted for 50.2% of all kidney transplants and 63.1% of all extrarenal transplants nationwide. There are more than 143.4 p.m.p. organ recipients in the Russian Federation. **Conclusion.** The geographic spread of transplant centers in the Russian Federation continues to expand. In 2023, five new centers were opened. Over the past year, the country has seen an increase in the number of effective donors and organ transplants. Because medical facilities still have untapped resources, the number of organ transplants performed is expected to rise. Moscow is the powerhouse of Russian transplantology. Shumakov National Medical Research Center of Transplantology and Artificial Organs and its branch perform 27.4% of the total number of organ transplants in the country. Among the successful regional initiatives, the following should be noted: the Republic of Tatarstan, Kemerovo Oblast (Kuzbass), St. Petersburg, Tyumen Oblast, Irkutsk Oblast. In the Russian Federation, pediatric transplant care is prioritized.

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

INTRODUCTION

The National Registry tracks current trends and developments in organ donation and transplantation in Russia under the auspices of a dedicated transplantology commission that was established in collaboration between the Russian Ministry of Health and the Russian Transplant Society. Previous reports have been published in 2009–2023 [1–14].

Information contained in the Registry has been previously presented in 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 mechanism for ensuring quality control and data collection integrity in the information system used to register donated human organs and tissues, donors and recipients. The system operates under executive order No. 355n of the Russian Ministry of Health, dated June 8, 2016.

In addition to statistical data for the reporting period, the Registry's annual reports include a systematic analysis with an assessment of the current status of transplantation care in the Russian Federation, as well as trends and prospects for future advancements in this branch of healthcare.

Since 2019, the National Registry has been used to monitor the implementation of the departmental target

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

Questionnaires are administered to the relevant officials at all transplant centers in the Russian Federation in order to gather data for the Registry. All of the data collected over the years from Russian regions, transplant centers and international registries is compared.

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

TRANSPLANT CENTERS

There are transplant centers in 38 federal subjects of the Russian Federation (see Fig. 1).

In order to comply with the regional principle in assessing the status and trends in transplant care and organ donation in the federal subjects of the Russian Federation, the activities of the Shumakov National Medical Research Center of Transplantology and Artificial Organs, Moscow (Shumakov Center) and its Volzhsky branch are further presented in the Registry separately as for two transplant centers.

In 2023, kidney transplantation (KTx) was performed in 50 centers, liver transplantation (LTx) in 34, heart transplantation (HTx) in 22, pancreas transplantation (PTx) in 2, and lung transplantation (LnTx) in 2 centers.

In 2023, 59 medical facilities carried out a variety of transplant interventions. Of these: 20 were federal institutions, including 13 institutions under the Russian Ministry of Health, 2 institutions under the Russian Ministry of Science and Higher Education, 4 institutions under the Federal Medical and Biological Agency, 1 institution under the Russian Ministry of Defense, and 39 are institutions run by federal subjects of the Russian Federation.

In the new territories of the Russian Federation, in 2023 there was one transplant center functioning in the Donetsk People's Republic (DPR) at the Donetsk Clinical Territorial Medical Association of the DPR Ministry of Health (known as Kalinin Republican Clinical Hospital since 2024), Donetsk. Three living-donor kidney transplants were performed last year.

In 2023, 3,057 organ transplants were performed in Russia, 281 were pediatric transplants (see Tables 1 and

Table 1

Organ donation and transplantation in the Russian Federation in 2023

Indicator	Number (units)
Organ donation	
Total number of organ donors	1274
Deceased donors	917
Living (related) donors	357
Organ transplantation	
Total number of organs transplanted	3057
share of pediatric transplants	281
Kidney	1817
from deceased donors	1620
from living-related donors	197
share of pediatric transplants	133
Liver	829
from deceased donor	669
from living-related donors	160
share of pediatric transplants	130
Heart	386
share of pediatric transplants	17
Heart-lung	2
Lungs	19
share of pediatric transplants	1
Pancreas	3

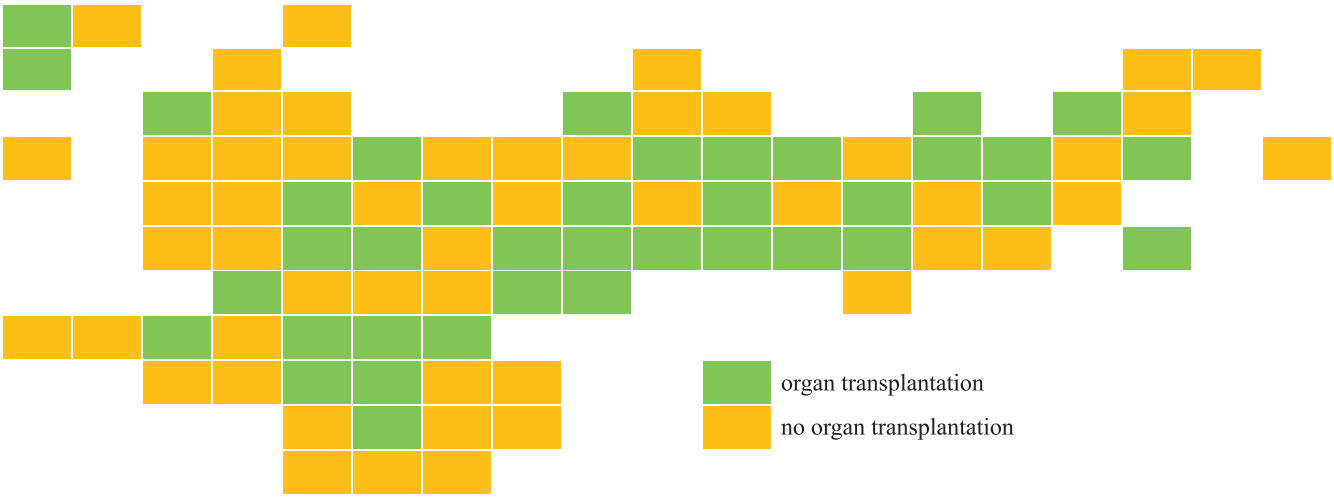

Fig. 1. Geographic spread of organ transplant centers in the Russian Federation in 2023

Table 2

Transplant activity in the Russian Federation in 2023

№	Transplant center, region, federal district	**Total**	**Kidney (total)**	Kidney (cadaver)	Kidney (living related)	**Liver (total)**	Liver (cadaver)	Liver (living related)	**Heart**	**Pancreas**	**Lungs**	**Heart-lungs**	**Small intestine**
1	2	3	4	5	6	7	8	9	10	11	12	13	14
1.1	Shumakov National Medical Research Center of Transplantology and Artificial Organs, **Moscow, Central Federal District**	**760**	**311**	215	96	**197**	95	102	**238**	**0**	**12**	**2**	**0**
1.2	Volzhsky Branch of Shumakov National Medical Research Center of Transplantology and Artificial Organs, **Volzhsky, Southern Federal District**	**77**	**48**	39	9	**17**	17	0	**12**	**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**	**76**	**76**	71	5	**0**	0	0	**0**	**0**	**0**	**0**	**0**
3	Russian Children's Clinical Hospital, **Moscow, Central Federal District**	**41**	**41**	35	6	**0**	0	0	**0**	**0**	**0**	**0**	**0**
4	Petrovsky National Research Centre of Surgery, **Moscow, Central Federal District**	**26**	**16**	8	8	**10**	0	10	**0**	**0**	**0**	**0**	**0**
5	Burnazyan Federal Medical and Biophysical Center, **Moscow, Central Federal District**	**45**	**8**	7	1	**37**	13	24	**0**	**0**	**0**	**0**	**0**
6	Bakulev Scientific Center of Cardiovascular Surgery, **Moscow, Central Federal District**	**2**	**0**	0	0	**0**	0	0	**2**	**0**	**0**	**0**	**0**
7	National Medical Research Center for Children's Health, **Moscow, Central Federal District**	**20**	**20**	9	11	**0**	0	0	**0**	**0**	**0**	**0**	**0**
8	Botkin Hospital, **Moscow, Central Federal District**	**173**	**118**	118	0	**51**	51	0	**4**	**0**	**0**	**0**	**0**
9	Sklifosovsky Research Institute of Emergency Care, **Moscow, Central Federal District**	**387**	**238**	237	1	**132**	128	4	**7**	**2**	**7**	**0**	**1**
10	Moscow Clinical Scientific Center, **Moscow, Central Federal District**	**51**	**1**	1	0	**50**	50	0	**0**	**0**	**0**	**0**	**0**
11	Vladimirsky Moscow Regional Research and Clinical Institute, **Moscow Oblast, Central Federal District**	**94**	**63**	63	0	**31**	31	0	**0**	**0**	**0**	**0**	**0**
12	Federal Clinical Center for High Medical Technologies, Federal Biomedical Agency (No. 119), **Moscow Oblast, Central Federal District**	**20**	**20**	16	4	**0**	0	0	**0**	**0**	**0**	**0**	**0**
13	Ivanovo Regional Clinical Hospital, **Ivanovo, Central Federal District**	**2**	**2**	2	0	**0**	0	0	**0**	**0**	**0**	**0**	**0**
14	St. Joasaphus Belgorod Regional Clinical Hospital, **Belgorod, Central Federal District**	**10**	**6**	6	0	**3**	3	0	**1**	**0**	**0**	**0**	**0**
15	Voronezh Regional Clinical Hospital No. 1, **Voronezh, Central Federal District**	**9**	**9**	8	1	**0**	0	0	**0**	**0**	**0**	**0**	**0**
16	Tula Regional Clinical Hospital, **Tula, Central Federal District**	**5**	**5**	4	1	**0**	0	0	**0**	**0**	**0**	**0**	**0**
17	Ryazan Regional Clinical Hospital, **Ryazan, Central Federal District**	**14**	**12**	11	1	**2**	2	0	**0**	**0**	**0**	**0**	**0**
18	Kursk Regional Multidisciplinary Clinical Hospital, **Kursk, Central Federal District**	**1**	**1**	0	1	**0**	0	0	**0**	**0**	**0**	**0**	**0**
19	Stavropol Regional Clinical Hospital, **Stavropol, North Caucasian Federal District**	**6**	**5**	4	1	**1**	1	0	**0**	**0**	**0**	**0**	**0**
20	Ochapovsky Regional Clinical Hospital No. 1, **Krasnodar, Southern Federal District**	**37**	**22**	19	3	**9**	8	1	**6**	**0**	**0**	**0**	**0**

Continuation table 2

1	2	3	4	5	6	7	8	9	10	11	12	13	14
21	Volzhsky Regional Center of Urology, **Volzhsky, Southern Federal District**	**1**	**1**	0	1	**0**	0	0	**0**	**0**	**0**	**0**	**0**
22	Rostov Regional Clinical Hospital, **Rostov-on-Don, Southern Federal District**	**65**	**40**	40	0	**18**	17	1	**6**	**1**	**0**	**0**	**0**
23	Russian Research Center of Radiology and Surgical Technologies, **St. Petersburg, Northwestern Federal District**	**17**	**0**	0	0	**17**	17	0	**0**	**0**	**0**	**0**	**0**
24	Almazov National Medical Research Centre, **St. Petersburg, Northwestern Federal District**	**33**	**0**	0	0	**0**	0	0	**33**	**0**	**0**	**0**	**0**
25	Pavlov University, **St. Petersburg, Northwestern Federal District**	**47**	**36**	31	5	**11**	11	0	**0**	**0**	**0**	**0**	**0**
26	St. Petersburg Research Institute of Emergency Medicine, **St. Petersburg, Northwestern Federal District**	**42**	**32**	32	0	**10**	10	0	**0**	**0**	**0**	**0**	**0**
27	Mariinskaya Hospital, **St. Petersburg, Northwestern Federal District**	**25**	**25**	25	0	**0**	0	0	**0**	**0**	**0**	**0**	**0**
28	St. Luke's Clinical Hospital, **St. Petersburg, Northwestern Federal District**	**29**	**29**	27	2	**0**	0	0	**0**	**0**	**0**	**0**	**0**
29	Kirov Military Medical Academy, **St. Petersburg, Northwestern Federal District**	**19**	**0**	0	0	**19**	19	0	**0**	**0**	**0**	**0**	**0**
30	Leningrad Regional Clinical Hospital, **St. Petersburg, Northwestern Federal District**	**37**	**36**	36	0	**1**	1	0	**0**	**0**	**0**	**0**	**0**
31	Volosevich First City Clinical Hospital, **Arkhangelsk, Northwestern Federal District**	**2**	**2**	2	0	**0**	0	0	**0**	**0**	**0**	**0**	0
32	Meshalkin National Medical Research Center, **Novosibirsk, Siberian Federal District**	**9**	**0**	0	0	**0**	0	0	**9**	**0**	**0**	**0**	**0**
33	State Novosibirsk Regional Clinical Hospital, **Novosibirsk, Siberian Federal District**	**75**	**33**	27	6	**42**	31	11	**0**	**0**	**0**	**0**	**0**
34	Research Institute for Complex Issues of Cardiovascular Diseases, **Kemerovo, Siberian Federal District**	**13**	**0**	0	0	**0**	0	0	**13**	**0**	**0**	**0**	**0**
35	Belyaev Kemerovo Regional Clinical Hospital, **Kemerovo, Siberian Federal District**	**68**	**64**	63	1	**4**	4	0	**0**	**0**	**0**	**0**	**0**
36	Irkutsk Regional Clinical Hospital, **Irkutsk, Siberian Federal District**	**69**	**39**	39	0	**28**	24	4	**2**	**0**	**0**	**0**	**0**
37	Altai Regional Clinical Hospital, **Barnaul, Siberian Federal District**	**20**	**20**	18	2	**0**	0	0	**0**	**0**	**0**	**0**	**0**
38	Federal Center for Cardiovascular Surgery, **Krasnoyarsk, Siberian Federal District**	**1**	**0**	0	0	**0**	0	0	**1**	**0**	**0**	**0**	**0**
39	Federal Siberian Research and Clinical Center, **Krasnoyarsk, Siberian Federal District**	**14**	**13**	13	0	**1**	1	0	**0**	**0**	**0**	**0**	**0**
40	Krasnoyarsk Clinical Hospital, **Krasnoyarsk, Siberian Federal District**	**38**	**19**	19	0	**10**	10	0	**9**	**0**	**0**	**0**	**0**
41	Sverdlovsk Regional Clinical Hospital No. 1, **Yekaterinburg, Ural Federal District**	**58**	**38**	38	0	**17**	17	0	**3**	**0**	**0**	**0**	**0**
42	Chelyabinsk Regional Clinical Hospital, **Chelyabinsk, Ural Federal District**	**30**	**18**	18	0	**8**	8	0	**4**	**0**	**0**	**0**	**0**
43	Regional Clinical Hospital No. 1, **Tyumen, Ural Federal District**	**36**	**32**	32	0	**2**	2	0	**2**	**0**	**0**	**0**	**0**
44	District Clinical Hospital, **Khanty-Mansiysk, Ural Federal District**	**16**	**11**	10	1	**4**	4	0	**1**	**0**	**0**	**0**	**0**
45	Samara State Medical University, **Samara, Volga Federal District**	**54**	**51**	50	1	**2**	2	0	**1**	**0**	**0**	**0**	**0**
46	Saratov State Medical University, **Saratov, Volga Federal District**	**7**	**7**	0	7	**0**	0	0	**0**	**0**	**0**	**0**	**0**
47	Regional Clinical Hospital, **Saratov, Volga Federal District**	**9**	**9**	9	0	**0**	0	0	**0**	**0**	**0**	**0**	**0**

End of table 2

1	2	3	4	5	6	7	8	9	10	11	12	13	14
48	Volga Regional Medical Center, **Nizhny Novgorod, Volga Federal District**	**30**	**20**	16	4	**10**	9	1	**0**	**0**	**0**	**0**	**0**
49	Republican Clinical Hospital, **Kazan, Volga Federal District**	**188**	**116**	112	4	**72**	71	1	**0**	**0**	**0**	**0**	**0**
50	Interregional Clinical Diagnostic Center, **Kazan, Volga Federal District**	**26**	**0**	0	0	**0**	0	0	**26**	**0**	**0**	**0**	**0**
51	Republican Clinical Hospital, **Ufa, Volga Federal District**	**44**	**38**	38	0	**6**	6	0	**0**	**0**	**0**	**0**	**0**
52	Republican Cardiology Clinic, **Ufa, Volga Federal District**	**5**	**0**	0	0	**0**	0	0	**5**	**0**	**0**	**0**	**0**
53	Sukhanov Federal Center for Cardiovascular Surgery, **Perm, Volga Federal District**	**1**	**0**	0	0	**0**	0	0	**1**	**0**	**0**	**0**	**0**
54	Perm Regional Clinical Hospital, **Perm, Volga Federal District**	**8**	**8**	6	2	**0**	0	0	**0**	**0**	**0**	**0**	**0**
55	Municipal Clinical Hospital for Emergency Medical Care No. 1, **Orenburg, Volga Federal District**	**24**	**24**	20	4	**0**	0	0	**0**	**0**	**0**	**0**	**0**
56	Republican Hospital No. 1 – National Center of Medicine, **Yakutsk, Far Eastern Federal District**	**2**	**1**	0	1	**1**	0	1	**0**	**0**	**0**	**0**	**0**
57	Semashko Republican Clinical Hospital, **Ulan-Ude, Far Eastern Federal District**	**4**	**4**	0	4	**0**	0	0	**0**	**0**	**0**	**0**	**0**
58	Primorsky Regional Clinical Hospital No. 1, **Vladivostok, Far Eastern Federal District**	**19**	**14**	14	0	**5**	5	0	**0**	**0**	**0**	**0**	**0**
59	Regional Clinical Hospital No. 1", **Khabarovsk, Far Eastern Federal District**	**16**	**15**	12	3	**1**	1	0	**0**	**0**	**0**	**0**	**0**
Total		**3057**	**1817**	**1620**	**197**	**829**	**669**	**160**	**386**	**3**	**19**	**2**	**1**

2). The number of organ transplants in Russia increased by 19.6% (+502) compared to 2022.

In 2023, between 192 (July) and 335 (April) organ transplants were performed per month (average of 255); see Fig. 2.

In the past year, between 114 and 192 KTx per month, 54 to 95 LTx and 19 to 46 HTx were performed.

Based on data obtained from the Federal Registry for High-Tech Medical Care, 2,683 (87.8%) organ transplant surgeries were performed in 2023, using funds from the mandatory health insurance system that were allocated for provision of high-tech medical care for organ transplantation; there were 2,186 (85.5%) of such surgeries in 2022; see Fig. 3. Another 374 (12.2%) organ transplants

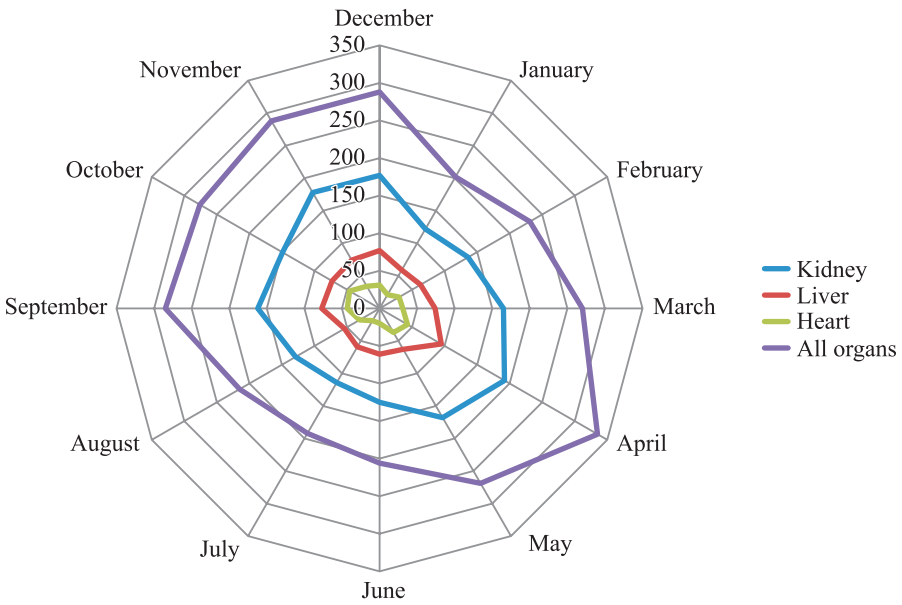

Fig. 2. Organ transplantation by months in 2023

were performed using funds from the federal subjects of the Russian Federation and from the federal budget (369 (14.5%) in 2022.

Thus, the increase in the number of organ transplants in 2023 became possible due to an adequate increase in funding from the mandatory health insurance system that was allocated for provision of high-tech medical care for organ transplantation.

Since 2010, when this indicator was included in the Registry, the number of organ transplants performed using the funds allocated for provision of high-tech medical care for organ transplant has increased 3.4-fold. Meanwhile, there has been a 29.6% increase in the number of organ transplants carried out with these funds.

The financial costs per unit of high-tech medical care for transplantation in 2023 were approved by the Government of the Russian Federation on December 29, 2022 via Resolution No. 2497.

ORGAN DONATION

In 2023, donor programs were implemented in 35 federal subjects of the Russian Federation.

Over the past year, two living donor programs were launched at:

– in Kursk Oblast (kidney),
– in Irkutsk Oblast (liver).

The number of effective deceased donors in 2023 was 917 or 6.3 p.m.p. (see Table 3). The number of effective deceased donors in the Russian Federation was 20.2% more than in 2022 (+154).

The proportion of effective deceased organ donors older than 60 years of age in 2023 was 22.3% (16.0% in 2022); see Fig. 4. At the same time, in 6 regions where

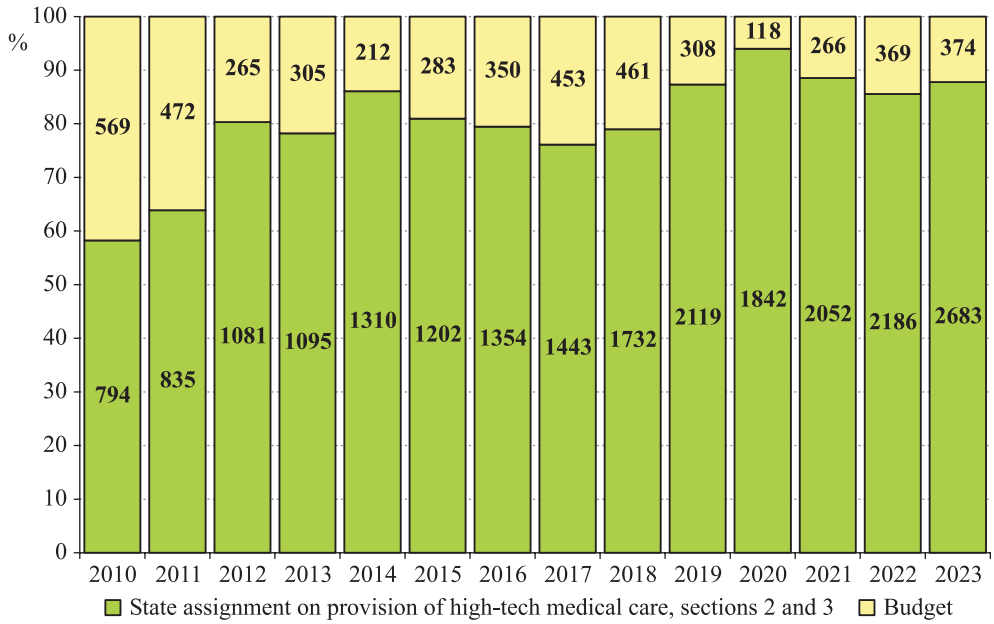

Fig. 3. Funding for organ transplantation in the Russian Federation between 2010 and 2023

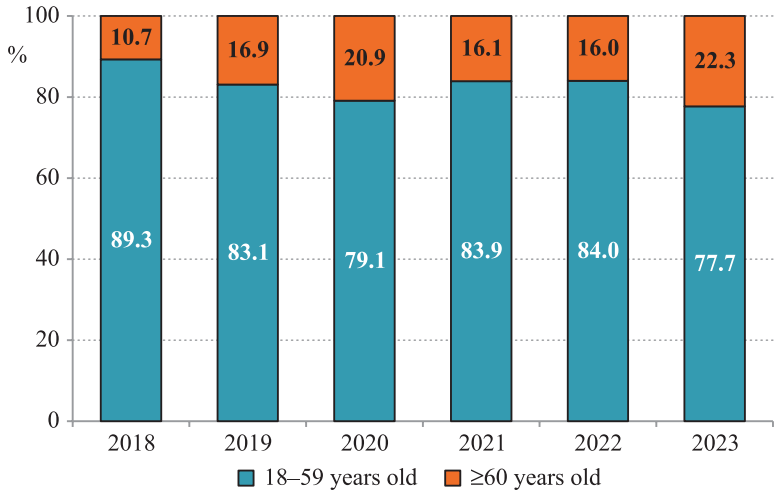

Fig. 4. Age structure of effective organ donors between 2018 and 2023

Table 3

Indicators associated with deceased organ donation across the Russian Federation in 2023

S/N	Region	Organ Donation Coordinating Center	Population (million)	Number of active donor bases	Donors (absolute, per million population)		including brain-dead donors (absolute, %)		including multi-organ donors (absolute, %)	
1	2	3	4	5	6	7	8	9	10	11
1	Moscow	Botkin Hospital	13.1	21	381	29.1	355	93.2	324	85.0
2	Moscow Oblast	Vladimirsky Moscow Regional Research and Clinical Institute	8.6	13	42	4.9	42	100.0	38	90.5
3	Ivanovo Oblast	Ivanovo Regional Clinical Hospital	1.0	1	2	2.0	2	100.0	2	100.0
4	Belgorod Oblast	St. Joasaph Belgorod Regional Clinical Hospital	1.5	1	3	2.0	3	100.0	3	100.0
5	Voronezh Oblast	Voronezh Regional Clinical Hospital No. 1	2.3	3	4	1.7	4	100.0	0	0.0
6	Tula Oblast	Tula Regional Clinical Hospital	1.5	1	5	3.3	4	80.0	4	80.0
7	Ryazan Oblast	Ryazan Regional Clinical Hospital	1.1	1	9	8.2	9	100.0	9	100.0
8	Krasnodar Krai	Ochapovsky Regional Clinical Hospital No. 1	5.8	1	13	2.2	12	92.3	9	69.2
9	Volgograd Oblast	Volzhsky Branch of Shumakov National Medical Research Center of Transplantology and Artificial Organs	2.5	3	15	6.0	15	100.0	11	73.3
10	Rostov Oblast	Rostov Regional Clinical Hospital	4.2	1	24	5.7	24	100.0	24	100.0
11	Stavropol Krai	Stavropol Regional Clinical Hospital	2.9	1	2	0.7	2	100.0	1	50.0
12	St. Petersburg	St. Petersburg Research Institute of Emergency Medicine	5.6	6	62	11.1	62	100.0	53	85.5
13	Leningrad Oblast	Leningrad Regional Clinical Hospital	2.0	1	18	9.0	18	100.0	14	77.8
14	Arkhangelsk Oblast	Volosevich First City Clinical Hospital	1.0	1	3	3.0	3	100.0	3	100.0
15	Novosibirsk Oblast	State Novosibirsk Regional Clinical Hospital	2.8	4	27	9.6	25	92.6	22	81.5
16	Kemerovo Oblast (Kuzbass)	Belyaev Kemerovo Regional Clinical Hospital	2.6	10	35	13.5	29	82.9	14	40.0
17	Irkutsk Oblast	Irkutsk Regional Clinical Hospital	2.3	2	26	11.3	26	100.0	24	92.3
18	Altai Krai	Altai Regional Clinical Hospital	2.1	1	9	4.3	9	100.0	2	22.2
19	Krasnoyarsk Krai	Krasnoyarsk Regional Clinical Hospital	2.8	3	13	4.6	12	92.3	9	69.2
20	Sverdlovsk Oblast	Sverdlovsk Regional Clinical Hospital No. 1	4.2	2	18	4.3	18	100.0	16	88.9
21	Chelyabinsk Oblast	Chelyabinsk Regional Clinical Hospital	3.4	1	13	3.8	13	100.0	10	76.9
22	Tyumen Oblast	Regional Clinical Hospital No. 1	1.6	3	17	10.6	17	100.0	3	17.6
23	Khanty-Mansi Autonomous Okrug – Yugra	District Clinical Hospital	1.7	3	5	2.9	5	100.0	4	80.0
24	Samara Oblast	Samara State Medical University	3.1	4	27	8.7	23	85.2	3	11.1
25	Saratov Oblast	Regional Clinical Hospital	2.4	1	9	3.8	9	100.0	7	77.8
26	Nizhny Novgorod Oblast	Volga Regional Medical Center	3.1	4	9	2.9	9	100.0	8	88.9
27	Republic of Tatarstan	Republican Clinical Hospital	4.0	3	68	17.0	68	100.0	60	88.2
28	Republic of Bashkortostan	Kuvatov Republican Clinical Hospital	4.1	6	20	4.9	20	100.0	8	40.0
29	Orenburg Oblast	Municipal Clinical Hospital for Emergency Medical Care No. 1	1.8	2	11	6.1	11	100.0	11	100.0
30	Primorsky Krai	Primorsky Regional Clinical Hospital No. 1	1.8	1	7	3.9	7	100.0	5	71.4
31	Perm Krai	Perm Regional Clinical Hospital	2.5	1	3	1.2	3	100.0	1	33.3

End of table 3

32	Khabarovsk Krai	Sergeev District Clinical Hospital	1.3	1	7	5.4	7	100.0	1	14.3
33	Departmental program of the Federal Biomedical Agency of the Russian Federation	Burnasyan Federal Medical Biophysical Center	-	2	1	-	1	100.0	1	100.0
34	Departmental program of the Federal Biomedical Agency of the Russian Federation	Federal Siberian Research and Clinical Center	-	3	9	-	9	100.0	4	44.4
		Total	**146.4**	**112**	**917**	**6.3**	**876**	**95.5**	**708**	**77.2**

the level of donor activity was higher than 10.0 effective donors p.m.p., their share was 25.8%.

Among other factors, expansion of organ transplant from donors over 60 years of age contributed to the rise in donor activity in 2023.

Donor activity per population of the regions implementing donor programs (100.7 million) amounted to 9.1 p.m.p. (see Tables 4 and 5).

Male donors made up 65.0% of the total, while female donors made up 35.0%.

Moscow posted the highest DA with 29.1 p.m.p. (26.3 in 2022). In the Republic of Tatarstan, DA was 17.0 effective donors p.m.p. (13.3). DA exceeded 10.0 p.m.p. in four more federal subjects of the Russian Federation – Kemerovo Oblast, Irkutsk Oblast, St. Petersburg and Tyumen Oblast.

In 2023, 25 federal subjects of the Russian Federation recorded increased DA, with Moscow, Moscow Oblast, St. Petersburg, Irkutsk Oblast and the Republic of Tatarstan posting the most dynamic growth (≥10 effective donors).

Five federal subjects of the Russian Federation witnessed a drop in DA; in Krasnodar Krai, Stavropol Krai and Belgorod Oblast, the decline was more severe than in other regions (by ≥25% decrease). However, given the consistently low DA in these regions, the decrease in 2023 did not have a significant impact on DA in the Russian Federation overall.

In 2023, 46.1% (423) of effective donors came from Moscow and Moscow Oblast alone (362, 47.4% in 2022). As a result, the Moscow agglomeration accoun-

Table 4

Rating of regions by donor activity in 2023

S/N	Federal Subject of the Russian Federation (Region)	Population in 2023 (million)	Donor count (per million population)	
			2023	2022
1	Moscow	13.1	29.1	26.3
2	Republic of Tatarstan	4.0	17.0	13.3
3	Kemerovo Oblast	2.6	13.5	15.8
4	Irkutsk Oblast	2.3	11.3	6.3
5	St. Petersburg	5.6	11.1	8.0
6	Tyumen Oblast	1.6	10.6	10.7
7	Novosibirsk Oblast	2.8	9.6	6.8
8	Leningrad Oblast	2.0	9.0	8.9
9	Samara Oblast	3.1	8.7	7.4
10	Ryazan Oblast	1.1	8.2	6.4
11	Orenburg Oblast	1.8	6.1	2.6
12	Volgograd Oblast	2.5	6.0	3.2
13	Rostov Oblast	4.2	5.7	5.0
14	Khabarovsk Krai	1.3	5.4	0.0
15	Republic of Bashkortostan	4.1	4.9	5.0
16	Moscow Oblast	8.6	4.9	3.8
17	Krasnoyarsk Krai*	2.8	4.6	3.4
18	Altai Krai	2.1	4.3	4.3
19	Sverdlovsk Oblast	4.2	4.3	2.3
20	Primorsky Krai	1.8	3.9	3.7
21	Saratov Oblast	2.4	3.8	2.9
22	Chelyabinsk Oblast	3.4	3.8	2.6
23	Tula Oblast	1.5	3.3	2.1
24	Arhangelsk Oblast	1.0	3.0	2.7
25	Nizhny Novgorod Oblast	3.1	2.9	2.2
26	Khanty-Mansi Autonomous Okrug – Yugra	1.7	2.9	1.8
27	Krasnodar Krai	5.8	2.2	3.0
28	Belgorod Oblast	1.5	2.0	3.3
29	Ivanovo Oblast	1.0	2.0	0.0
30	Voronezh Oblast	2.3	1.7	1.3
31	Perm Krai	2.5	1.2	1.0
32	Stavropol Krai	2.9	0.7	1.1
	Russia (85 federal subjects of the Russian Federation)	146.4	6.3	5.2

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

Table 5

Deceased organ donors (effective donors) between 2006 and 2023

S/N	Region	2006	2007		2008		2009		2010		2011		2012		2013		2014		2015		2016		2017		2018		2019		2020		2021		2022		2023	
		Donor count	Donor count	Year-over-year change (abs.)	Donor count	Year-over-year change (abs.)	Donor count	Year-over-year change (abs.)	Donor count	Year-over-year change (abs.)	Donor count	Year-over-year change (abs.)	Donor count	Year-over-year change (abs.)	Donor count	Year-over-year change (abs.)	Donor count	Year-over-year change (abs.)	Donor count	Year-over-year change (abs.)	Donor count	Year-over-year change (abs.)	Donor count	Year-over-year change (abs.)	Donor count	Year-over-year change (abs.)	Donor count	Year-over-year change (abs.)	Donor count	Year-over-year change (abs.)	Donor count	Year-over-year change (abs.)	Donor count	Year-over-year change (abs.)
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37
1	Moscow	87	126	+39	135	+9	136	+1	151	+15	135	–16	111	–24	125	+14	151	+26	142	–9	183	+41	195	+12	218	+23	277	+59	263	–14	298	+35	332	+34	381	+49
2	Moscow Oblast	24	45	+21	59	+14	52	–7	71	+19	82	+11	61	–21	56	–5	51	–5	44	–7	39	–5	75	+36	68	–7	41	–27	21	–20	36	+15	30	–6	42	+12
3	Belgorod Oblast		2	+2	3	+1	2	–1	5	+3	6	+1	3	–3	1	–2	2	+1	5	+3	4	–1	4	0	4	0	4	0	2	–2	2	0	5	+3	3	–2
4	Voronezh Oblast	6	2	–4	8	+6	2	–6	0	–2	1	+1	6	+5	6	0	5	–1	7	+2	4	–3	1	–3	8	+7	8	0	4	–4	3	–1	3	0	4	+1
5	Tula Oblast																										2	+2	3	+1	4	+1	3	–1	5	+2
6	Ryazan Oblast																								2	+2	13	+11	6	–7	11	+5	7	–4	9	+2
7	Ivanovo Oblast																														1	+1	0	–1	2	+2
8	Krasnodar Krai						3	+3	39	+36	52	+13	42	–10	41	–1	23	–18	25	+2	24	–1	19	–5	20	+1	23	+3	13	–10	13	0	17	+4	13	–4
9	Volgograd Oblast	5	0	–5	11	+11	15	+4	16	+1	17	+1	19	+2	15	–2	18	+3	8	–10	8	0	9	+1	9	0	10	+1	10	0	10	0	8	–2	15	+7
10	Rostov Oblast																		1	+1	7	+6	13	+6	19	+6	21	+2	18	–3	21	+3	21	0	24	+3
11	Stavropol Krai																								2	+2	3	+1	13	+10	5	–8	3	–2	2	–1
12	St. Petersburg	30	45	+15	47	+2	47	0	41	–6	34	–7	22	–12	13	–9	23	+10	31	+8	29	–2	31	+2	34	+3	53	+19	25	–28	25	0	43	+18	62	+19
13	Leningrad Oblast	12	8	–4	11	+3	11	0	13	+2	10	–3	10	0	10	0	9	–1	7	–2	12	+5	11	–1	15	+4	7	–8	11	+4	12	+1	17	+5	18	+1
14	Arkhangelsk Oblast																								5	+5	5	0	1	–4	1	0	3	+2	3	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	19	+4	27	+8
16	Kemerovo Oblast	16	13	–3	18	+5	18	0	22	+4	12	–10	26	+14	26	0	31	+5	28	–3	34	+6	22	–12	30	+8	40	+10	27	–13	28	+1	41	+13	35	–6
17	Irkutsk Oblast				4	+4	6	+2	10	+4	9	–1	8	–1	6	–2	9	+3	4	–5	3	–1	2	–1	7	+5	16	+9	16	0	8	–8	15	+7	26	+11
18	Omsk Oblast	10	15	+5	13	–2	19	+6	19	0	14	–5	11	–3	14	+3	16	+2	11	–5	4	–7	4	0	3	–1	2	–1	2	0	0	–2	0	0	0	0
19	Altai Krai														3	+3	5	+2	4	–1	4	0	8	+4	8	0	8	0	9	+1	7	–2	10	+3	9	–1
20	Krasnoyarsk Krai																3	+3	6	+3	18	+12	27	+9	16	Note	13	–3	10	–3	12	+2	10	–2	13	+3
21	Sverdlovsk Oblast	14	13	–1	12	–1	13	+1	14	+1	15	+1	14	–1	18	+4	23	+5	18	–5	15	–3	22	+7	24	+2	24	0	6	–18	14	+8	10	–4	18	+8

End of table 5

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37
22	Chelyabinsk Oblast								6	+6	2	–4	7	+5	6	–1	10	+4	9	–1	11	+2	8	–3	4	–4	4	0	3	–1	3	0	9	+6	13	+4
23	Tyumen Oblast																						4	+4	13	+9	13	0	5	–8	8	+3	16	+8	17	+1
24	Khanty-Mansi Autonomous Okrug – Yugra																						3	+3	4	+1	5	+1	3	–2	2	–1	3	+1	5	+2
25	Samara Oblast	4	17	+13	24	+7	18	–6	20	+2	21	+1	19	–2	21	+2	20	–1	18	–2	26	+8	28	+2	23	–5	25	+2	24	–1	24	0	23	–1	27	+4
26	Saratov Oblast														4	+4	7	+3	7	0	7	0	7	0	8	+1	10	+2	0	–10	6	+6	7	+1	9	+2
27	Nizhny Novgorod Oblast						7	+7	11	+4	12	+1	10	–2	8	–2	12	+4	10	–2	11	+1	10	–1	12	+2	12	0	5	–7	7	+2	7	0	9	+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	52	+17	68	+16
29	Republic of Bashkortostan								2	+2	7	+5	14	+7	18	+4	19	+1	14	+5	20	+6	22	+2	20	–2	24	+4	18	–6	21	+3	20	–1	20	0
30	Orenburg Oblast																		3	+3	8	+5	9	+1	8	–1	11	+3	1	–10	4	+3	5	+1	11	+6
31	The Republic of Sakha (Yakutia)																				2	+2	4	+2	4	0	3	–1	0	–3	0	0	1	+1	0	–1
32	Primorsky Krai																														3	+3	7	+4	7	0
33	Perm Krai																																1	+1	3	+2
34	Khabarovsk Krai																																		7	+7
35	Burnasyan Federal Medical and Biophysical Center, Moscow														6	+6	11	+5	14	+3	16	+2	9	–7	5	–4	1	–4	1	0	3	+2	2	–1	1	–1
36	Burnasyan Federal Medical and Biophysical Center, Krasnoyarsk																								24	Note	16	–8	8	–8	10	+2	13	+3	9	–4
	TOTAL in the Russian Federation	**225**	**300**	**+75**	**364**	**+64**	**381**	**+17**	**487**	**+106**	**470**	**–17**	**412**	**–58**	**420**	**+8**	**465**	**+45**	**434**	**–31**	**499**	**+53**	**565**	**+78**	**639**	**+74**	**732**	**+93**	**564**	**–168**	**652**	**+88**	**763**	**+111**	**917**	**+154**

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

ted for 39.6% of the 2023 growth in DA, while the other regions of the country accounted for 60.4%.

There were 876 effective brain-dead donors, making up 95.5% of the entire pool of effective donors (see Fig. 5). In 24 federal subjects of the Russian Federation, the centers worked only with brain-dead donors. For the first time, no donor programs had a brain-dead donor percentage lower than 80.0%.

There were 708 multi-organ procurements in 2023, accounting for 77.2% of the total number of procurements. In 20 federal subjects, the percentage of multi-organ procurements was ≥70.0%.

In Voronezh Oblast, Kemerovo Oblast, Altai Krai, Tyumen Oblast, Samara Oblast, Republic of Bashkortostan, Perm Krai, and Khabarovsk Krai, the percentage of multi-organ donors is less than 50.0%, indicating underutilization of donor resources.

In 2023, Moscow and Moscow Oblast alone accounted for 362 multi-organ donors (51.1% of the total number of multi-organ donors) in the country (294 and 38.5% in 2022).

An average of 2.9 organs were procured from a single donor in 2023 (2.8 in 2022). Donor kidney utilization rate was 88.3% (compared to 87.4% in 2022).

In 2023, the number of organ procurements (kidney, part of liver) from living related donors was 357 organ procurements or 28.0% of the total 1,274 procurements (386, 33.6% in 2022).

KIDNEY TRANSPLANTATION

A total of 1,817 KTx were performed in 2023 (see Fig. 6).

Fifty centers performed KTx. The number of KTx increased by 16.3% (+255) in comparison to the year 2022. A new living-related-donor KiT program was launched in Kursk Oblast (Kursk Regional Multidisciplinary Clinical Hospital, Kursk).

In 2023, there were 1,620 deceased-donor KTx and 197 living-related-donor KTx (see Fig. 6).

The KTx centers that carried out the highest number of kidney transplants in 2023 are listed in Table 6 and Fig. 7.

The rating demonstrates the leadership and advancement of the transplant programs at top transplant centers in the capital city Moscow, which is a result of effective efforts by the Moscow Coordinating Center for Organ Donation.

However, in other federal subjects of the Russian Federation, KTx centers showed a significant increase in transplant activity in 2023: St. Petersburg (+40), Republic of Tatarstan (+33), Moscow Oblast (+24), Irkutsk Oblast (+21), Sverdlovsk Oblast (+18), Volgograd Oblast (+12), Samara Oblast (+10) and others.

Eight KTx centers performed more than 50 surgeries in 2023. They are Shumakov Center (311), Sklifosovsky Research Institute for Emergency Medicine (238), Botkin Hospital (118), Republican Clinical Hospital, Kazan (116), Lopatkin Research Institute of Urology (76), Kuzbass Regional Clinical Hospital (64), Vladimirsky Moscow Regional Research Clinical Institute (63), and Samara State Medical University (51). Eleven transplant centers performed from 30 to 49 operations during the year and another 12 centers performed between 15 and 29. The remaining 19 (38%) performed less than 15 kidney transplants in the year.

In 2023, 30 transplant facilities (60%) performed related-donor KTx, carrying out a total of 197 transplants. Just four kidney transplant centers performed 7

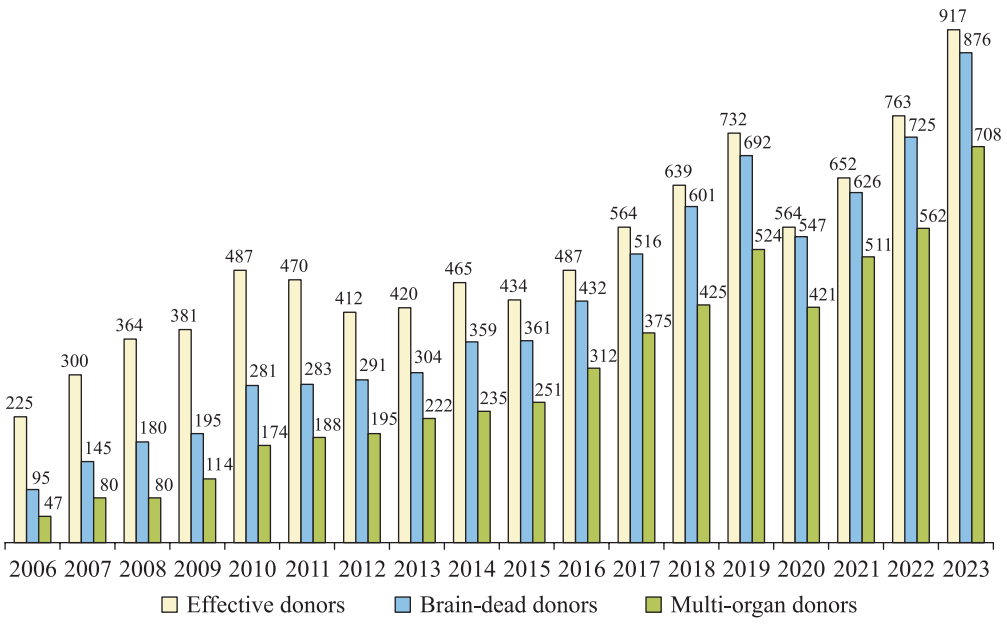

Fig. 5. Structure of effective organ donors in the Russian Federation in 2006–2023

or more operations in the year – Shumakov Center (96) and its branch (9), National Medical Research Center for Children's Health (11), Petrovsky National Research Centre of Surgery (8), and St. Petersburg State Medical Center (8). At the same time, Shumakov Center and its branch performed 53.3% (105) of all related-donor kidney transplants in the Russian Federation. The average utilization of living kidney donation in 2023 was 10.8% of the total number of kidney transplants (14.6% in 2022).

Pediatric KTx (minors ≤17 years of age) in 2023 were performed in 6 centers, a total of 133 transplants were performed (118 in 2022). Among the institutions involved were Shumakov Center (61), Russian Children's

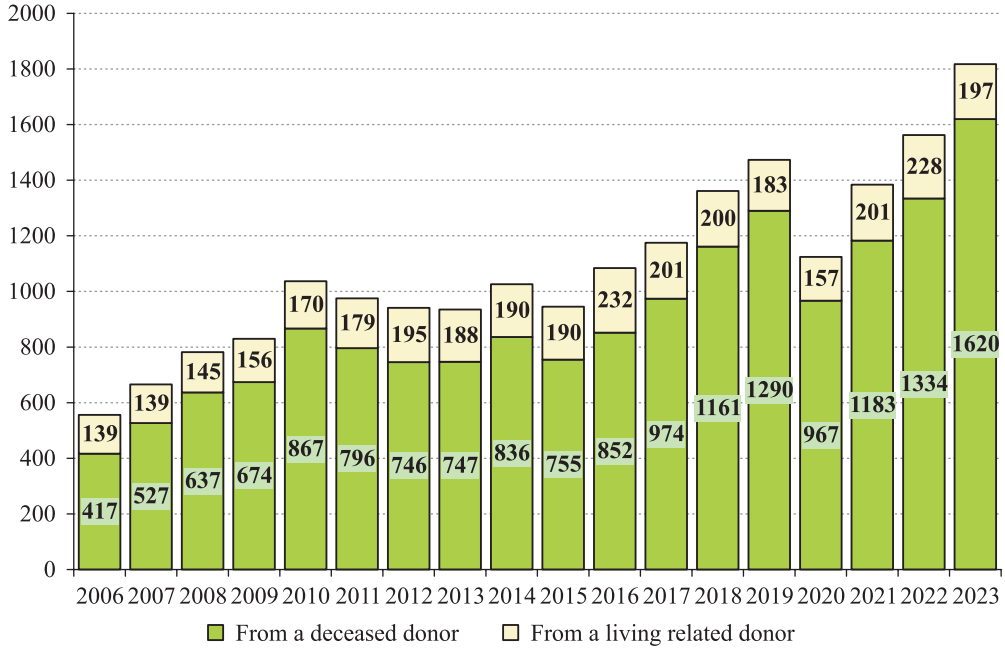

Fig. 6. Kidney transplantation in the Russian Federation in 2006–2023

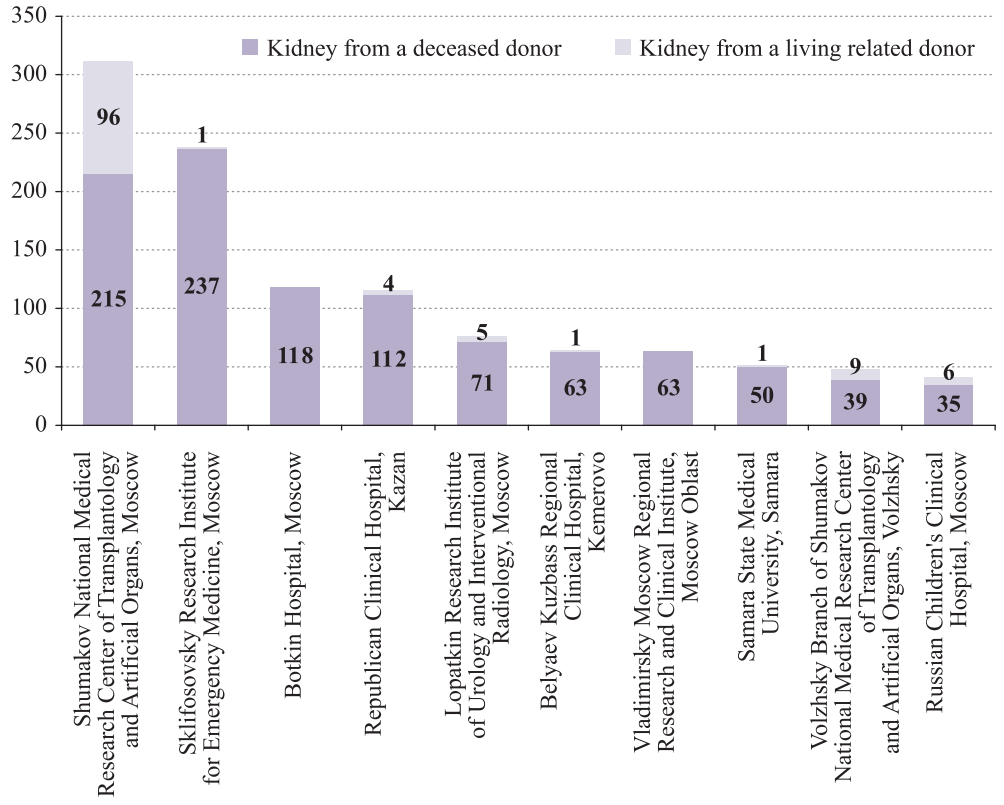

Fig. 7. Leading centers by number of kidney transplants performed

Table 6

Leading centers by number of kidney transplants performed

Rank	Leading institutions in terms of number of kidney transplants performed	Number of kidney transplants in 2023
1	Shumakov National Medical Research Center of Transplantology and Artificial Organs, Moscow	311
2	Sklifosovsky Research Institute for Emergency Medicine, Moscow	238
3	Botkin Hospital, Moscow	118
4	Republican Clinical Hospital, Kazan	116
5	Lopatkin Research Institute of Urology and Interventional Radiology, a branch of the National Medical Research Radiological Center, Moscow	76
6	Belyaev Kuzbass Regional Clinical Hospital, Kemerovo	64
7	Vladimirsky Moscow Regional Research and Clinical Institute, Moscow Oblast	63
8	Samara State Medical University, Samara	51
9	Volzhsky Branch of Shumakov National Medical Research Center of Transplantology and Artificial Organs, Volzhsky	48
10	Russian Children's Clinical Hospital, Moscow	41
	TOTAL	**1126**
	62.0% of the total number of kidney transplants in the Russian Federation (1817)	

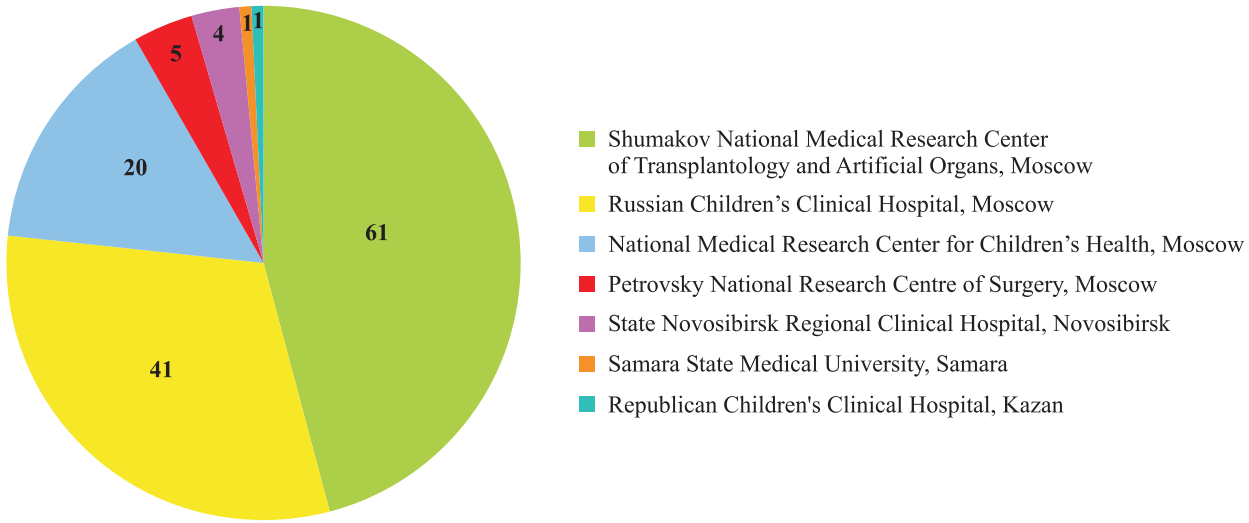

Fig. 8. Pediatric kidney transplantation in the Russian Federation in 2023

Clinical Hospital (41), and National Medical Research Center for Children's Health (20); see Fig. 8.

EXTRARENAL ORGAN TRANSPLANTATION

Of the 388 heart transplants performed in 2023, 17 were pediatric transplants and 2 were heart-lung transplants (at Shumakov Center). Compared to 2022, the number of HTx increased by 25.3% (+78).

Twenty-two centers performed heart transplants. A new HTx program was launched in Perm Krai (Sukhanov Federal Center for Cardiovascular Surgery).

Shumakov Center (240), including its Volzhsky branch (12), accounted for 64.9% of the total number of heart transplants in the Russian Federation (252, including 2 heart-lung transplants). The HTx program at this center continues to drive the level of availability of this type of transplant care in the country.

The thoracic organ transplant centers that performed the highest number of heart-lung transplants in 2023 are listed in Table 7 and Fig. 9.

Apart from Shumakov Center (Moscow), more than 10 heart transplants in the Russian Federation were performed at Almazov National Medical Research Centre, St. Petersburg (33), the Interregional Clinical Diagnostic Center in Kazan (26), the Research Institute for Complex Issues of Cardiovascular Diseases in Kemerovo (13) and the branch of Shumakov Center in Volzhsky (12).

It should be noted that the number of heart transplants performed increased from 8 to 26 in the Republic of Tatarstan, from 2 to 12 at the branch of Shumakov Center in Volzhsky (12) and from 28 to 33 in St. Petersburg.

Meshalkin National Medical Research Center (Novosibirsk), Regional Clinical Hospital (Krasnoyarsk), Sklifosovsky Research Institute for Emergency Medici-

ne (Moscow), Ochapovsky Regional Clinical Hospital No. 1 (Krasnodar), Rostov Regional Clinical Hospital (Rostov-on-Don), and Republican Clinical Hospital (Ufa) were the other six transplant centers that conducted 5 to 9 heart transplants in 2023. The remaining 11 (50%) performed less than 5 HTx in the year.

Lung transplants were performed in 2 transplant centers in 2023. A total of 19 lung transplants and 2 heart-lung transplants were performed: 12 lung transplants and 2 heart-lung transplants at Shumakov Center, 7 LnTx were performed at Sklifosovsky Research Institute for Emergency Medicine.

A total of 829 liver transplants, including 130 pediatric transplants, were performed in 2023. Liver transplants were performed at 34 centers. Compared to 2022, the number of liver transplants rose by 170 or 25.8% (659 in 2022).

Three new liver transplant programs were launched in 2023: from a living-related donor at Irkutsk Regional Clinical Hospital and from a deceased donor at Regional Clinical Hospital No. 1 in Khabarovsk and Kuzbass Regional Clinical Hospital in Kemerovo.

In 2023, two transplant centers – Shumakov Center (197) and Sklifosovsky Research Institute for Emergency

Table 7

Leading centers by number of heart transplants performed

Rank	Leading institutions in terms of number of heart transplants performed	Number of heart transplants in 2023
1	Shumakov National Medical Research Center of Transplantology and Artificial Organs, Moscow	240*
2	Almazov National Medical Research Centre, St. Petersburg	33
3	Interregional Clinical and Diagnostic Center, Kazan	26
4	Research Institute for Complex Issues of Cardiovascular Diseases, Kemerovo	13
5	Volzhsky Branch of Shumakov National Medical Research Center of Transplantology and Artificial Organs, Volzhsky	12
6	Meshalkin National Medical Research Center, Novosibirsk	9
7	Krasnoyarsk Regional Clinical Hospital, Krasnoyarsk	9
	TOTAL	**342**
	88.1% of the total number of heart transplants performed in the Russian Federation (388)	

*, including two heart-lung transplants.

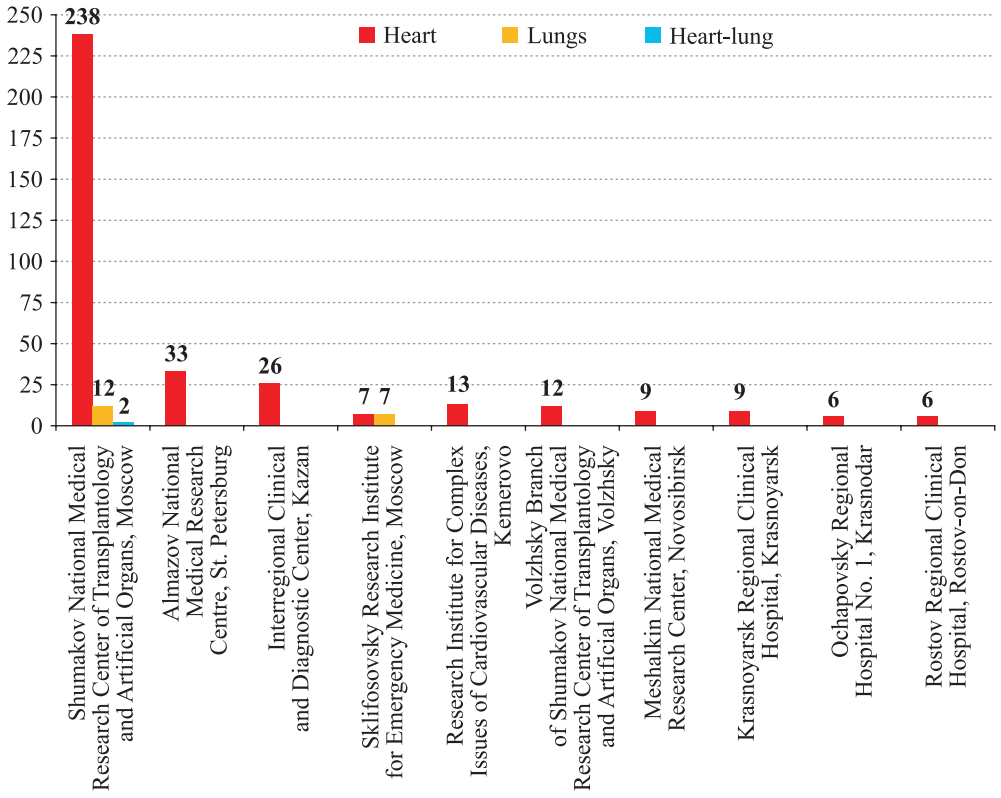

Fig. 9. Leading centers by number of heart transplants performed

Medicine (132) – performed over 100 liver transplants. Four other transplant centers – Republican Clinical Hospital, Kazan (72), Botkin Hospital (51), Moscow Clinical Scientific Center (50), and State Novosibirsk Regional Clinical Hospital (42) – carried out 40 or more liver transplants each. Nine centers performed 15 to 40 liver transplants.

The remaining 19 (57.6%) performed less than 15 liver transplants in the year.

The transplant centers that performed the highest number of liver transplants in 2023 are listed in Table 8 and Fig. 10.

The rating demonstrates the leadership of transplant programs at top transplant centers in the capital city Moscow, which is a result of effective efforts by the Moscow Coordinating Center for Organ Donation and the use of living-donor liver transplant. It is important to highlight the successful transplant programs in the Republic of Tatarstan, Sverdlovsk Oblast, Irkutsk Oblast, and

Table 8

Leading centers by number of liver transplants performed

Rank	Leading institutions in terms of number of liver transplants performed	Number of liver transplants in 2023
1	Shumakov National Medical Research Center of Transplantology and Artificial Organs, Moscow	197
2	Sklifosovsky Research Institute of Emergency Care, Moscow	132
3	Republican Clinical Hospital, Kazan	72
4	Botkin Hospital, Moscow	51
5	Moscow Clinical Scientific Center, Moscow	50
6	State Novosibirsk Regional Clinical Hospital, Novosibirsk	42
7	Burnazyan Federal Medical and Biophysical Center, Moscow	37
8	Vladimirsky Moscow Regional Research and Clinical Institute, Moscow Oblast	31
9	Irkutsk Regional Clinical Hospital, Irkutsk	28
	TOTAL	**640**
	77,2% of the total number of liver transplants performed in the Russian Federation (829)	

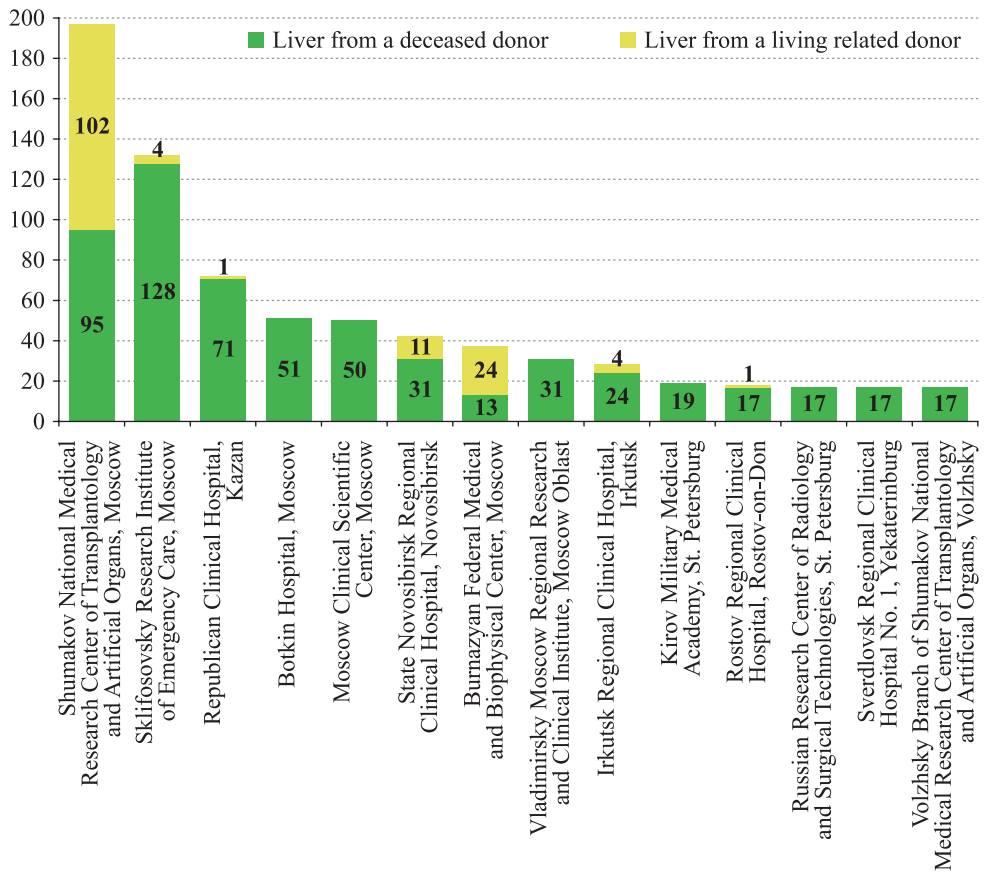

Fig. 10. Leading centers by number of liver transplants performed

the Volzhskiy branch of Shumakov Center. Additionally, the pediatric living related LTx program at Shumakov Center (Moscow) plays should also be noted.

A total of 160 related liver transplant procedures were performed at 11 centers (32.4%). Shumakov Center (102), Burnazyan Federal Medical and Biophysical Center (24), State Novosibirsk Regional Clinical Hospital in Novosibirsk (11), and Petrovsky National Research Centre of Surgery (10), were the only four liver transplant centers that conducted ten or more procedures during the year. Meanwhile, the Shumakov Center performed 63.7% of all related liver transplants in the Russian Federation. The average utilization of living kidney donation in 2023 was 19.3% (24.0% in 2022) of the total number of liver transplants.

In 2023, 130 pediatric liver transplants (mostly in tender-age children) were performed. Pediatric LTx were carried out at 4 centers: Shumakov Center (117) and its Volzhsky branch (1), Petrovsky National Research Centre of Surgery (9), State Novosibirsk Regional Clinical Hospital in Novosibirsk (2), and Irkutsk Regional Clinical Hospital (1). Shumakov Center accounted for 90.8% of all the pediatric liver transplants performed. The pediatric program at this facility continues to drive the level of availability of this type of transplant care in the nation.

In 2023, pancreas transplants were performed at 2 transplant centers: Sklifosovsky Research Institute for Emergency Medicine (2) and Rostov Regional Clinical Hospital in Rostov-on-Don (1). Three pancreas transplant surgeries were performed (10 in 2022), all of them being kidney-pancreas transplants. One small intestinal transplant was also done at Sklifosovsky Research Institute for Emergency Medicine.

Thus, there were 1,240 extrarenal transplants in 2023 or 40.6% of the total number of 3,057 transplants (993, 38.9% in 2022). Transplant facilities in the Moscow agglomeration alone accounted for 63.1% (783) of extrarenal organ transplants in 2023.

Over the follow-up period beginning in 2006, the number of extrarenal organ transplant procedures in the Russian Federation increased by 1,134 (11.7-fold); see Figs. 11 and 12.

The number of organ transplants carried out in the Russian Federation between 2006 and 2023 is presented in Table 9.

ORGAN TRANSPLANT RECIPIENTS

As of December 2022, there were about 21,000 organ transplant recipients in the Russian Federation; see Table 10.

Over 9 years of follow-up, the number of organ recipients in the Russian Federation increased 2.5-fold; the number of kidney recipients is estimated at 14,258 (97.4 p.m.p.); liver recipients, 4,644 (31.7 p.m.p.); heart recipients, 2,084 (14.2 p.m.p.).

CONCLUSION

The Russian Federation saw a 19.6% (+502) increase in organ transplants in 2023 compared to 2022. At the same time, there was a:

– 16.3% (+255) increase in kidney transplants performed;
– 25.3% (+78) increase in heart transplants;
– 25.8% (+170) increase in liver transplants.

The number of effective deceased donors in the Russian Federation rose by 20.2% (+154) compared to 2022.

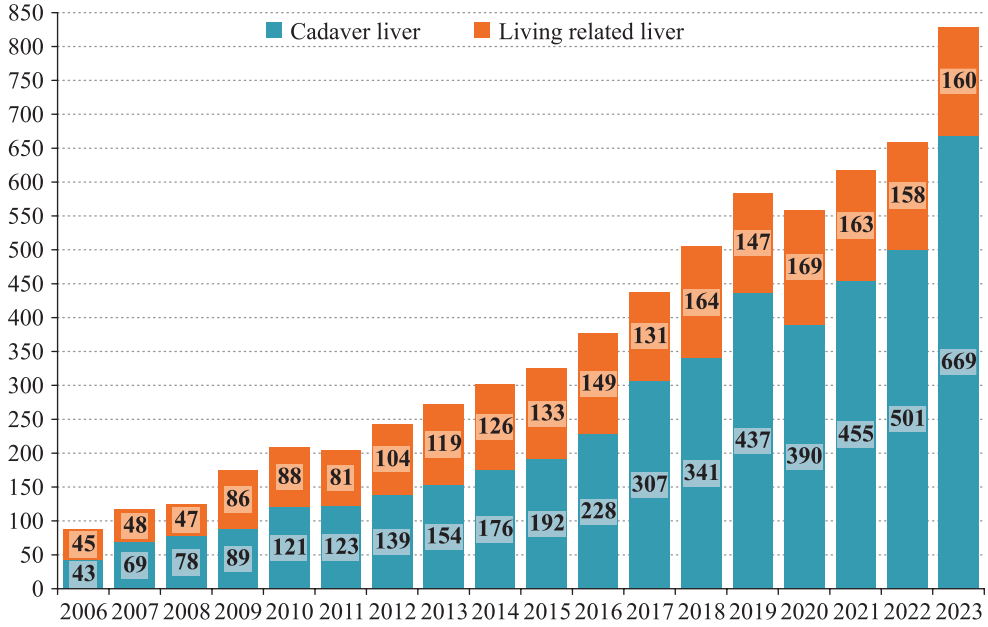

Fig. 11. Liver transplantation in the Russian Federation between 2006 and 2023

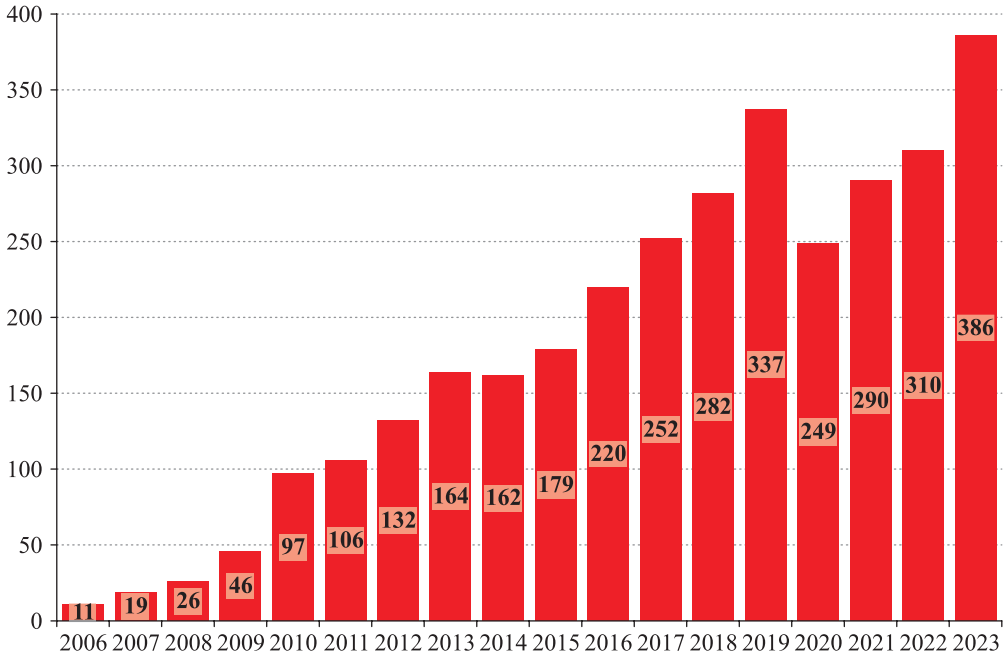

Fig. 12. Heart transplantation in the Russian Federation between 2006 and 2023

In 2023, the primary goals and trends in the evolution of organ donation and transplantation across federal subjects in the Russian Federation remained the same and did not lose their relevance:

– expansion of the geographic footprint and increase in the number of transplant centers;
– efficient identification of patients in need and their placement on the organ transplant waiting list;
– increase in the number of deceased organ donors in proportion to available donor resources, increase in the proportion of multi-organ donors;
– increase in organ transplants in proportion to the needs of the population;
– a focus on providing transplant care to the pediatric population;
– 100.0% coverage of medical screening, including medication supply, for transplant recipients.

Five new organ donation and transplantation programs were opened in 2023:

– living-related-donor kidney transplant was performed in Kursk Oblast (Kursk Regional Multidisciplinary Clinical Hospital);
– heart transplant was performed in Perm Krai (Sukhanov Federal Center for Cardiovascular Surgery);
– two living-related-donor kidney transplants were performed in Irkutsk Oblast (Irkutsk Regional Clinical Hospital);
– deceased-donor liver transplant was performed in Khabarovsk Krai (Regional Clinical Hospital No. 1);
– four deceased-donor liver transplants were carried out in the Kemerovo Oblast (Kuzbass Regional Clinical Hospital).

Moscow remains the undisputed leader in terms of organ donation and transplant in the Russian Federation, demonstrating a high level of donor and transplant activity in global practice. Other regional programs showing high activity include the Republic of Tatarstan, Kemerovo Oblast (also known as Kuzbass), Irkutsk Oblast, St. Petersburg, Tyumen Oblast and the branch of Shumakov Center (Volgograd Oblast, Volzhsky).

In Shumakov Center and its Volzhsky branch accounted for 27.4% of all organ transplant procedures performed in the country; 53.3% of related kidney transplants; 61.8% of heart transplants; 63.7% of related liver transplants.

The number of patients on waiting lists across transplant centers remains at approximately the same level, increasing when new facilities and organ transplant programs are established anywhere in the Russian Federation, as well as when the transplant activity at these centers increases.

Results from donor programs in Moscow (29.1 effective donors p.m.p.), the Republic of Tatarstan (17.0), and Kemerovo Oblast (also known as Kuzbass, 13.5) indicate a high potential for increasing the number of deceased donors in other federal subjects in the nation with proper organization of this activity, including control by the executive authorities of federal subjects in the nation in the field of health care; adequate financial support for medical activities related to organ donation for transplantation; active engagement with expanded criteria donors, including donors over 60 years of age.

The average proportion of effective brain-dead organ donors in the Russian Federation is above 95.0%, while that of multi-organ donors is above 75.0%. This indi-

Table 9

Organ transplantation in the Russian Federation between 2006 and 2023

S/N	Organ	2006	2007		2008		2009		2010		2011		2012		2013		2014		2015		2016		2017		2018		2019		2020		2021		2022		2023	
		Absolute number	Absolute number	Year-over-year change	Absolute number	Year-over-year change	Absolute number	Year-over-year change	Absolute number	Year-over-year change	Absolute number	Year-over-year change	Absolute number	Year-over-year change	Absolute number	Year-over-year change	Absolute number	Year-over-year change	Absolute number	Year-over-year change	Absolute number	Year-over-year change	Absolute number	Year-over-year change	Absolute number	Year-over-year change	Absolute number	Year-over-year change	Absolute number	Year-over-year change	Absolute number	Year-over-year change	Absolute number	Year-over-year change	Absolute number	Year-over-year change
1	Kidney (total)	556	666	+110	782	+116	830	+48	1037	+207	975	–62	941	–34	935	–6	1026	+91	945	–81	1084	+139	1175	+91	1361	+186	1473	+112	1124	–349	1382	+258	1562	+180	1817	+255
2	*from a deceased donor*	417	527	+110	637	+110	666	+29	867	+201	796	–71	746	–50	747	+1	836	+89	755	–81	852	+97	974	+122	1161	+187	1290	+129	967	–323	1183	+216	1334	+151	1620	+286
3	*from a living related donor*	139	139	0	145	+6	156	+11	170	+14	179	+9	195	+16	188	–7	190	+2	190	0	232	+42	201	–31	200	–1	183	–17	157	–26	201	+44	228	+27	197	–31
4	Liver (total)	88	117	+29	125	+8	175	+50	209	+34	204	–5	243	+39	272	+29	302	+30	325	+23	378	+53	438	+60	505	+67	584	+79	559	–25	618	+59	659	+41	829	+170
5	*from a deceased donor*	43	69	+26	78	+9	89	+11	121	+32	123	+2	139	+16	154	+15	176	+22	192	+16	229	+37	307	+78	341	+34	437	+96	390	–47	455	+65	501	+46	669	+168
6	*from a living related donor*	45	48	+3	47	–1	86	+39	88	+2	81	–7	104	+23	119	+15	126	+7	133	+7	149	+16	131	–18	164	+33	147	–17	169	+22	163	–6	158	–5	160	+2
7	Heart	11	19	+8	26	+7	46	+20	97	+51	106	+9	132	+26	164	+32	162	–2	179	+17	220	+41	252	32	282	+30	335	+53	249	–86	290	+41	308	+18	386	+78
8	Pancreas	6	11	+5	9	–2	8	–1	19	+11	14	–5	23	+9	14	–9	19	+5	12	–7	6	–6	6	0	17	+11	10	–7	16	+6	10	–6	10	0	3	–7
9	Lungs	1	0	–1	0	0	1	+1	1	0	6	+5	5	–1	10	+5	12	+2	14	+2	16	+2	25	+9	25	0	23	–2	9	–14	13	+4	14	+1	19	+5
10	Heart-lung										2	+2	2	0	1	–1	0	–1	0	0	0	0	0	0	3	+3	2	–1	2	0	2	0	1	–1	2	+1
11	Small intestine														1	+1	1	0	0	–1	0	0	0	0	0	0	0	0	1	+1	1	0	0	–1	1	+1
	Total	662	813	+151	942	+129	1060	+118	1363	+303	1307	–56	1345	+38	1400	+55	1522	+122	1485	–37	1704	+219	1896	+192	2193	+297	2427	+234	1960	–467	2318	+358	2555	+237	3057	+502

Table,10

Number of organ transplant recipients in the Russian Federation between 2013 and 2023

ICD-10 code	Patient count in the Registry (persons)																			
	2013	2014		2015		2016		2017		2018		2019		2020		2021*		2022*		2023*	
		Absolute	YoY change (%)	Absolute	YoY change (%)	Absolute	YoY change (%)	Absolute	YoY change (%)	Absolute	YoY change (%)	Absolute	YoY change (%)	Absolute	YoY change (%)	Absolute	YoY change (%)	Absolute	YoY change (%)	Absolute	YoY change (%)
Z94.0 Kidney transplant status	6651	7502	12.8	8164	8.8	9063	11.0	9658	6.6	10,851	12.4	11,880	9.5	12,563	5.7	12,969	–	13,514	–	14,258	–
Z94.1 Heart transplant status	416	520	25.0	639	22.9	803	25.7	952	18.6	1164	22.3	1355	16.4	1524	12.5	1687	–	1855	–	2084	–
Z94.2 Lung transplant status	2	3	50.0	4	33.3	5	25.0	8	60.0	28	250.0	26	–7.1	24	–7.7	–	–	–	–	–	–
Z94.4 Liver transplant status	1150	1406	22.3	1649	17.3	1948	18.1	2152	10.5	2632	22.3	3032	15.2	3489	15.1	3820	–	4165	–	4644	–
Z94.8 Other transplanted organ and tissue status (bone marrow, intestines, pancreas)	334	467	39.8	654	40.0	808	23.5	909	12.5	1135	24.9	1344	18.4	1497	11.4	–	–	–	–	–	–
TOTAL	8553	9898	15.7	11,110	12.2	12,627	13.7	13,679	8.3	15,810	15.6	17,637	11.6	19,097	8.3	–	–	–	–	–	–

* *Note*. The number of organ transplant recipients is estimated as it is calculated from the figures of the previous year based on data on the number of organ transplants in 2021-2023 and data on the average patient survival.

cates that there is efficient donor resource utilization in most federal subjects in Russia that are involved in organ donation activities. The failure to achieve the above values in Tyumen Oblast, Kemerovo Oblast (Kuzbass), Republic of Bashkortostan, and Samara Oblast should be viewed by healthcare managers and specialists as the unsatisfactory outcome of their activities. Based on this information, they should develop and implement a plan of appropriate measures to improve the efficiency of donor programs in their respective regions.

The number of organ transplants in the Russian Federation continues to increase systematically, and the existing capacities at healthcare facilities that operate on both donors and recipients allow for the potential expansion of transplant care, provided sufficient funding is available and waiting lists and donor support are in place.

The necessary conditions have been created in the Russian Federation to prioritize the provision of transplant care to the pediatric population. All identified children in need of organ transplantation receive such care as soon as possible, usually at federal centers (Shumakov Center, Russian Children's Clinical Hospital, National Medical Research Center for Children's Health, Petrovsky National Research Centre of Surgery) and at a number of regional medical facilities. The efficiency of locating and directing such patients from the federal subjects of the Russian Federation will determine whether or not the number of pediatric transplants continues to rise. To address this issue, the Shumakov Center is in continuous interaction with tertiary children's hospitals and with chief freelance pediatricians collaborating with healthcare executive authorities at federal subjects of the Russian Federation.

In 2023, specialists at Shumakov Center regularly went on field trips in order to study and analyze how transplant care in the regions are organized. They also held daily telemedicine consultations, educational conferences and methodological seminars. These measures created favorable conditions and the necessary focus for further implementation of transplant technologies across Russia. Also, healthcare authorities and medical institutions involved in the provision of organ transplant care and in medical activities related to organ donation for transplantation, received methodological assistance while developing regional road maps (development plans) for transplant care. These plans have shown to be a useful tool for multi-year planning of donor and transplant initiatives.

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RISK FACTORS AND PREDICTORS OF RECURRENT VARICEAL BLEEDING IN CIRRHOTIC PATIENTS AWAITING TRANSPLANTATION

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Objective: to identify the risk factors and predictors of recurrent variceal hemorrhage in cirrhotic patients awaiting liver transplantation (LT). **Materials and methods.** A comparative retrospective study was conducted in 51 patients with decompensated cirrhosis, who were on the waiting list for LT. Demographic, clinical and laboratory parameters, MELD-Na score, Child–Turcotte–Pugh score, hepatic encephalopathy grade, ascites grade, class of varicose veins, number of consecutive variceal ligations, as well as manometric study with calculation of intrahepatic venous pressure gradient index in groups of patients with (n = 39) and without recurrent bleeding (n = 12) were analyzed. The proportions of patients in different groups were compared by the Kaplan–Meier method with determination of the logarithmic test (Log-Rank). The accumulated risks in the compared groups were estimated using the mathematical model of proportional hazards (Cox regression) in univariate and multivariate analysis. **Results.** Within 60 months from the beginning of follow-up and simultaneous prophylaxis by combination of non-selective beta-blockers and endoscopic variceal ligation (EVL), 39 out of 51 patients (75.6%) developed recurrent bleeding. Analysis revealed significant differences (risk factors for recurrent bleeding): creatinine levels, MELD-Na score, hepatic encephalopathy grade, mean hepatic venous pressure gradient (HVPG) and its level >14 mmHg. By the Kaplan–Meier method with the Log-Rank test, it was established that the proportion of patients without recurrent bleeding was significantly higher in the group of patients with HVPG ≤14 mmHg than in the group with HVPG >14 mmHg (p = 0.027). **Conclusion.** The main independent predictor of variceal rebleeding is HVPG >14 mm Hg, which increases the risk by 3.837 times if the gradient value is changed by 1 mm. The second independent predictor is higher hepatic encephalopathy grade: if the grade increases by one, the risk of recurrent hemorrhage increases 1.8 times.

Keywords: liver transplantation, ascites, recurrent variceal bleeding, endoscopic variceal ligation, nonselective beta-blockers, risk factors, independent predictors.

INTRODUCTION

Recurrent variceal bleeding (RVB) is a serious and potentially life-threatening complication of cirrhosis [1]. Garcia-Tsao et al. [2] found that a sizable percentage of patients were still at risk of experiencing recurrent bleeding (RB) even after a period of stabilization following the development of the first variceal bleeding episode. When emergency measures are not taken to stop variceal hemorrhage, early RB develops over the next 2–3 days after the initial episode, and the frequency reaches 60% [3]. Within a period of up to 1 year, the risk of RB is 60% [4] or 29–57% within 2 years following the first bleeding episode, despite prophylactic measures [5].

It is unclear exactly what mechanisms lead to the rupture of esophageal varices. Portal venous pressure (PVP) has been shown to be the primary determinant of the progression of variceal bleeding. It is known that PVP exceeds 10 mmHg and variceal rupture occurs at PVP >12 mmHg in patients with varices without bleeding [3].

Is it possible to predict RB and mortality based on various prognostic models that are built using risk factors and predictors? When analyzing literature sources, we encountered a great deal of discrepancy in both the factual identification of these factors and the identification methodology itself. In our opinion, risk factors and predictors of an event are different epidemiological characteristics. A risk factor is an event, circumstance or characteristic that is present in a subject, that is common in sufferers of a particular disease, or characterizing a certain state (phase or stage) in the development of the disease. A predictor is a circumstance, characteristic or event that occurs while an action is taking place, that favors one particular outcome (positive or negative) [6].

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Analysis of scholarly publications show that studies by Moitinho et al. [7] and Abraldes et al. [8] were the first to identify predictors of early RVB and mortality from bleeding. Using multiple logistic regression analysis as a predictive model, the authors of this research found that a hepatic venous pressure gradient (HVPG) ≥20 mmHg was an independent predictor of RB in patients awaiting liver transplantation (LT). A Cox proportional-hazards model was used by Ripoll et al. to establish that each 1-mmHg increase in HVPG predicts a 3% increase in waitlist mortality at 19 months [9].

A contemporary study by Ardevol et al. [10] included 369 patients with cirrhosis followed up for 46 months after the first bleeding episode. Forty-five patients (12%) had early rebleeding early (within 6 weeks), 74 patients (20%) had late rebleeding (more than 6 weeks), and 250 (68%) had no rebleeding. Using Cox proportional-hazards model to assess the risks of developing early and late recurrent bleeding, the presence of ascites or hepatic encephalopathy (HE), MELD score >12 and HVPG >20 mmHg were found to be significant predictors of developing early RB. Mortality risk was significantly higher in the early RB group versus late RB (HR = 0.476, 95% CI = 0.318–0.712, p<0.001). Adjustment for baseline risk factors [MELD and Child–Turcotte–Pugh (CTP)], led to the conclusion that early RB is an **independent predictor** of mortality risk (HR = 1.58, 95% CI = 1.02–2.45; p = 0.04). The authors concluded by justifying early implantation of a transjugular intrahepatic portosystemic shunt (pre-emptive TIPS) within 72 hours after the onset of the first bleeding episode in order to prevent early RB and reduce patient mortality.

Objective: to identify the risk factors and predictors of recurrent variceal hemorrhage in cirrhotic patients awaiting LT.

MATERIALS AND METHODS

A comparative retrospective study was conducted in 51 patients with decompensated cirrhosis who were on the LT waitlist (LTWL) between 2018 and 2023. These patients developed RVB following secondary prophylaxis through a combination of non-selective beta-blocker (NSBB) and endoscopic variceal ligation (EVL).

The inclusion criteria were: first episode of variceal hemorrhage during stay in the LTWL in patients with decompensated cirrhosis, cirrhosis of any etiology – virus-related (HBV- or HCV-) cirrhosis, alcohol-related cirrhosis, or cirrhosis of mixed etiology (virus-related and alcohol-related), complete abstinence for at least 3 months (confirmed by narcologists) prior to inclusion in the LTWL, CTP classes B and C.

Exclusion criteria: hepatocellular carcinoma or any other tumors, any infectious diseases, portal vein thrombosis, intolerance or contraindications to NSBB (bradyarrhythmia, bronchial asthma, obstructive pulmonary disease, low mean arterial pressure (mAP)), and diabetes mellitus.

A continuously updated electronic database of patients enrolled in the LTWL at the Center for Surgery and Donation Coordination (CSDC), Rostov Regional Clinical Hospital, was the basis for subsequent analysis of demographic, clinical, and laboratory parameters, after approval of the study by the Local Ethics Committee. Follow-up of patients was conducted by specialists at CSDC. When patients were enrolled in the LTWL, they were examined, and this included laboratory and instrumental tests, the frequency of which depended on the patients' condition. Full blood count and biochemical tests were performed when patients were in a stable condition; hemostasis, MELD-Na score and CTP class were analyzed. Laboratory parameter studies were conducted every three months, and abdominal ultrasounds were conducted every six months. For unstable patients awaiting LT, laboratory and instrumental studies were performed when indicated.

The Baveno VI Consensus Workshop [[11] and the World Gastroenterology Association (WGO) [12] guidelines served as the basis for screening all patients with varices that are at a high risk of hemorrhage (medium-sized and large-sized varices) via esophagogastroduodenoscopy (EGD).

The severity of diuretic-responsive and diuretic-resistant ascites was graded according to the International Club of Ascites (ICA) criteria [13]. In addition to the ICA criteria [13], the Cirrhotic Ascites Severity (CIRAS) scale [14], including clinical and laboratory criteria, was used to characterize diuretic-resistant ascites. When a patient has a CIRAS score of 5–6, the diagnosis of diuretic-resistant ascites was considered quite definite [14].

Hepatic encephalopathy (HE) was graded according to the West Haven criteria [15].

Mean arterial pressure (mAP) was determined by the formula: mAP = (DP) + $^1/_3$ (SP – DP), where SP is systolic pressure, and DP is diastolic pressure [27].

Patients received diuretics; those with diuretic-resistant ascites had 1 to 5 paracentesis. In accordance with recognized expert guidelines, antiviral medication with nucleoside analogs or a combination of direct-acting antivirals was given if HBV- and HCV-associated cirrhosis was identified [17].

In accordance with the Baveno VII guidelines, all patients were treated with first-line therapy with a combination of propranolol or carvedilol and EVL to prevent RB [18].

Propranolol was given to 17 patients at a starting dose of 40 mg/day and the maximum was 240 mg/day. Carvedilol was administered to 34 patients at an initial dose of 6.25 mg/day and the maximum was 25 mg/day. All patients were monitored for heart rate (HR), blood pressure (BP) and mean BP (mAP). A decrease in these indicators served as the basis for dosage modification.

The EVL procedure was performed under EGD sedation using a variceal ligation kit. EVL started at the gastroesophageal junction and continued proximally using multiple rubber ligatures (2 to 4 or more), the number of which was determined by variceal size. Repeated ligations were carried out 1 month after the first procedure, and this manipulation was repeated until all varices satisfying the criteria for emergency therapy were completely obliterated [11, 12]. Repeated EGDs at three-month intervals were used to track variceal obliteration. Repeated ligation procedures were performed for recurrence (appearance of new varices).

The development of re-bleeding during first-line therapy (combination of propranolol or carvedilol + EVL) was considered as a failure of bleeding prophylaxis, which served as a justification for TIPS.

Esophageal manometry (EM) was carried out in all patients in order to clarify the relationship between re-bleeding during prophylaxis with first-line therapy and the magnitude of HVPG.

Following transjugular access, the J-shaped end of a standard angiographic guidewire was placed in the inferior vena cava (IVC) slightly above the hepatic vein orifices. A balloon catheter with a pressure transducer at the end (Edwards Lifesciences, USA) was used for EM. The pressure in the right hepatic vein (RHV) was measured using a catheter, the tip of which was freely placed 1–3 cm from its confluence with the IVC, obtaining a free hepatic venous pressure (FHVP).

When the balloon was inflated and the pressure curve stabilized, we measured the wedged hepatic venous pressure (WHVP). Three measurements were taken in order to get the arithmetic mean of WHVP. Occlusion of the RHV with a catheter was monitored via angiography (sinusoidal graph) after administration of 2–5 mL of contrast agent in the absence of its reflux or washout.

HVPG was calculated using the formula: HVPG = WHVP – FHVP.

The IBM SPSS Statistics software package (version 23) was used to analyze the obtained data for statistical studies. At the first stage, the type of distribution of the obtained variables of the studied samples was determined using the Kolmogorov–Smirnov test and the Lilliefors significance level. For normal distribution of variables, the arithmetic mean (M) was calculated, and the standard deviation (SD) was determined. The significance of differences between the compared values was determined by Student's t test using a significance threshold of $p < 0.05$. For non-normal distribution, the analysis of variables included determination of median (Me) with interquartile range (IQR, the interval between 25th and 75th percentiles). When conducting pairwise comparisons of dependent variables, the Wilcoxon signed-rank test used in nonparametric analysis was used to determine the significance of differences between them. Pearson's chi-squared test was used to compare independent variables For a small sample, the variables were compared by calculating the Mann–Whitney U test. Analysis of variance was performed using ANOVA test. Conjugacy tables were used to analyze qualitative parameters (frequencies of variables and their shares in %); for small samples, Fisher's exact test was used to assess the significance of the relationship between two variables.

The Kaplan–Meier method was used to compare the percentage of patients in different groups. The significance of differences between the compared curves (patient proportions) was determined by calculating the logarithmic test [Log-Rank (Mantel-Cox)].

Comparative assessment of accumulated risks in groups was carried out using a mathematical model of proportional risks (Cox regression) in univariate and multivariate analysis. The risk of occurrence of the tested event (HR) was calculated and the 95% confidence interval (CI) for this indicator was determined. The quality of the model used was determined by estimating the maximum likelihood (log-likelihood, –2LL). The condition of multivariate Cox proportional hazards regression analysis (absence of linear relationship between independent variables, which creates redundancy in the model) was verified by constructing a correlation matrix.

RESULTS

The patients, who were enrolled in the study for up to 60 months of being in the LTWL and received secondary prophylaxis through a combination of EVL and NSBB, were divided into two groups. The first group consisted of patients (n = 39) who developed re-bleeding despite prophylaxis, and the second group (n = 12) consisted of patients who had no recurrent bleeding.

Demographic, clinical, laboratory parameters, as well as MELD-Na and CTP scores in the groups of patients with and without RB during their stay in the LTWL are presented in Table 1.

As can be seen from the table presented, hemoglobin level, creatinine content, MELD-Na and CTP scores, HE grade, number of consecutive variceal ligations, mean HVPG and mean HVPG >14 mmHg, which were higher in the RB group than in the non-RB group, reached significant differences between the compared groups.

In the first group of patients with RB before initiation of prophylaxis, 14 patients (27.5%) had a single bleeding episode, and 25 patients (72.5%) had >1 bleeding episode before initiation of prophylactic therapy. As a result of prophylaxis, 2 out of 39 (5.1%) patients developed two RB episodes, and 37 out of 39 patients (94.9%) developed ≥3 RB episodes.

RB developed within 1 week after the first bleeding episode in 3 (7.7%) out of 39 patients, within 4 weeks in 7 (17.9%) out of 39 patients, and within 6 weeks in 10 out of 39 patients (25.6%) awaiting LT.

We compared RB incidence in the two groups of patients differing in HVPG.

The first group consisted of patients with HVPG ≤14 mmHg (n = 8), and the second group with HVPG >14 mmHg (n = 31). In group 1 and 2, 4 (50%) and 26 (83.9%) patients, respectively, experienced re-bleeding, difference between groups (p = 0.046).

Using the Kaplan–Meier method, it was established that the proportion of patients without re-bleeding was significantly greater in the group of patients with HVPG ≤14 mmHg than in the group of patients with HVPG >14 mmHg (Log Rank = 0.027) (Fig. 1).

We used survival analysis to predict the risk of recurrent hemorrhage for patients awaiting LT. This analysis is used in biomedical research to predict mortality, disease recurrence, or recovery, or any other outcomes relative to the time of their occurrence [19]. The influence of independent variables (predictors) on RB risk was assessed using a mathematical Cox proportional hazards model with calculation of the risk of an adverse event (Hazard Risk; HR) and determination of the 95% CI.

For this purpose, we used univariate and multivariate analysis of the mathematical Cox proportional hazards model (Table 2).

When **univariate analysis** was applied, a model with one independent variable was created with calculation of the hazard ratio (HR), confidence interval (CI) and assessment of the significance of the effect on the development of adverse event (rebleeding) for each predictor. All independent variables (predictors), significantly influencing the development of RB in univariate analysis, are presented in the first part of Table 2.

As can be seen from Table 2, in the univariate analysis of the mathematical Cox proportional hazards model, independent variables that significantly influence the development of an adverse outcome (rebleeding) were identified: creatinine level, MELD-Na score, number of consecutive variceal ligations, HE grade, categorical HVPG (HVPG ≤14 mmHg and HVPG >14 mmHg), HVPG ≤14 mmHg, HVPG >14 mmHg.

Multivariate analysis involved the creation of a model designed to assess the independent contribution of several predictors simultaneously, while determining the significance of their influence on RB. The role of all simultaneously acting significant predictors in RB development in multivariate analysis is shown in the second part of Table 2. Here, we used the forced-entry method, in which all variables are simultaneously entered into the model. Statistically significant predictors, determined by univariate analysis (taking into account each predictor separately), as well as known risk factors for RB, regardless of their influence in the univariate analysis, were selected for inclusion in the multivariate

Table 1

Comparative characteristics of patients with and without recurrent bleeding (normal and non-normal distribution)

Indicator	RB (n = 39), M ± SD	No RB (n = 12), M ± SD	p value
Normal distribution (M ± SD)			
Age	51.26 ± 10.21	46.83 ± 7.48	0.17
Hemoglobin (g/L)	86.32 ± 21.07	116.23 ± 20.35	**0.049**
White blood cells ($\times 10^9$/L)	4.58 ± 1.72	4.30 ± 1.68	0.62
Plasma albumin (g/L)	30.54 ± 2.96	30.75 ± 2.95	0.83
Creatinine (μmol/L)	131.54 ± 10.96	102.33 ± 11.02	**0.042**
INR	1.96 ± 0.45	1.78 ± 0.39	0.19
MELD-Na (points)	25.56 ± 4.57	15.49 ± 5.21	**0.031**
HE grade (points)	1.97 ± 0.99	1.25 ± 1.14	**0.034**
mAP (mmHg)	89.26 ± 11.32	86.08 ± 7.79	0.37
HVPG (mmHg)	16.54 ± 2.86	13.25 ± 1.14	**0.001**
HVPG ≤14 (mmHg)	10.02 ± 1.24	13.65 ± 1.17	0.35
HVPG >14 (mmHg)	18.61 ± 1.12	13.13 ± 1.14	**0.04**
Number of consecutive EVLs	4.46 ± 0.60	1.33 ± 0.35	**0.04**
Non-normal distribution (Me; IQR)			
Platelets ($\times 10^9$/L)	91.0 (67.0–111.0)	117.00 (65.6–168.25)	0.45
Bilirubin (μmol/L)	76.0 (65.0–85.0)	78.0 (74.75–148.00)	0.17
Na (mmol/L)	131.0 (130.0–134.0)	131.5 (129.250–134.25)	0.84
CTP (points)	14.0 (8.0–16.2)	8.0 (8.0–12.5)	**0.04**
Ascites grade	2.0 (1.0–3.0)	2.5 (2.00–4.0)	0.19
Esophageal varices grade	3.0 (3.0–3.0)	3.0 (2.25–3.0)	0.39

Note: RB, recurrent bleeding; EVL, endoscopic variceal ligation; INR, International normalized ratio; MELD-Na, Model for End-Stage Liver Disease-Sodium; CTP, Child–Turcotte–Pugh; Na, sodium; HE, hepatic encephalopathy; mAP, mean arterial pressure; HVPG, hepatic venous pressure gradient.

analysis model, which is allowed in the construction of this regression model [19, 20].

As shown in Table 2, a hazard ratio (HR) >1.0 was significant for HE grade, HVPG (cat.) and HVPG >14 mmHg, which allows us to consider these factors as having an independent effect on RB risk. HR shows how many times the risk of an outcome changes if the predictor value is changed by one. So, applying it to the

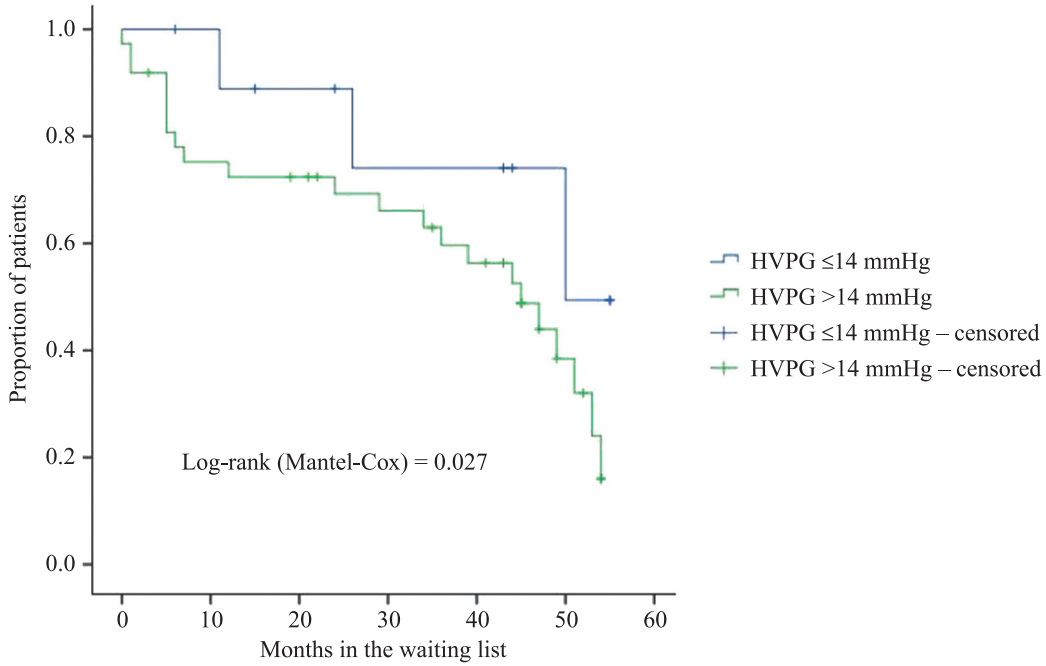

Fig. 1. Proportion of patients without bleeding and with recurrent bleeding after prophylaxis by endoscopic ligation and non-selective beta-blockers, depending on HVPG (Kaplan–Meier method with Log-Rank test)

Table 2

Univariate and multivariate analysis of predictors associated with recurrent bleeding after secondary prophylaxis by a combination of endoscopic variceal ligation and nonselective beta-blockers

Variables	Univariate analysis		Multivariate analysis	
	HR (CI)	p-value	HR (CI)	p value
Age	1.005 (0.966–1.044)	0.82	–	–
Platelets (×10^9/L)	1.002 (0.998–1.007)	0.34	–	–
White blood cells (×10^9/L)	1.086 (0.970–1.300)	0.37	–	–
Plasma albumin (g/L)	1.012 (0.900–1.138)	0.85	–	–
INR	2.045 (0.957–4.369)	0.06	–	–
Bilirubin (μmol/L)	1.002 (0.996–1.007)	0.53	–	–
Creatinine (μmol/L)	1.002 (0.996–1.007)	**0.03**	0.924 (0.929–1.063)	0.85
Na (mmol/L)	1.091 (0.988–1.205)	0.08	–	–
Hemoglobin (g/L)	1.014 (0.921–1.143)	0.79	–	–
MELD-Na (points)	1.236 (1.096–1.394)	**0.01**	1.172 (0.597–2.301)	0.64
CTP (points)	1.312 (1.070–1.234)	**0.003**	1.027 (0.852–1.238)	0.78
Ascites grade	0.651 (0.462–0.919)	0.23	0.591 (0.412–0.848)	**0.004**
Esophageal varices grade	0.780 (0.373–1.631)	0.51	1.362 (0.317–5.847)	0.68
Number of consecutive EVLs	0.881 (0.526–1.473)	**0.04**	0.512 (0.224–1.173)	0.11
HE grade (points)	1.698 (1.192–2.420)	**0.003**	1.800 (1.141–2.841)	**0.012**
mAP (mmHg)	0.989 (0.860–1.019)	0.46	–	–
HVPG (mmHg; cat.)*	1.237 (1.015–1.522)	**0.012**	1.324 (1.050–1.675)	**0.007**
HVPG ≤14 (mmHg)	0.563 (0.312–0.789	**0.007**	0.613 (0.436–0.863)	**0.005**
HVPG >14 (mmHg)	3.563 (3.131–4.075)	**0.009**	3.837 (2.995–4.235	**0.002**

Note: * – variable including two HVPG categories: ≤14 and >14 mmHg. HR, hazard ratio; EVL, endoscopic variceal ligation; MELD-Na, Model for End-Stage Liver Disease-Sodium; INR, International normalized ratio; CTP, Child–Turcotte–Pugh; Na, sodium; HE, hepatic encephalopathy; mAP, mean arterial pressure; HVPG, hepatic venous pressure gradient.

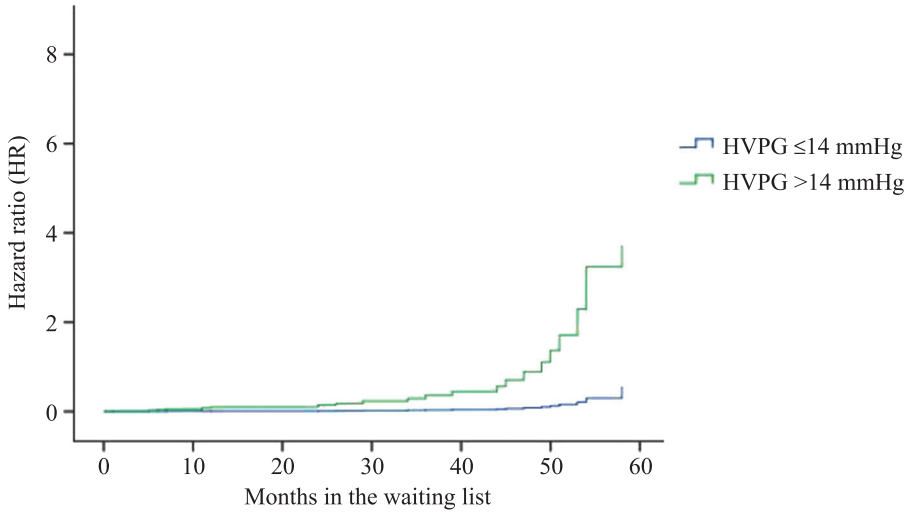

Fig. 2. Hazard ratio (HR) for recurrent bleeding as a function of time and magnitude of the categorical variable (≤14 mmHg; >14 mmHg)

results obtained, we can say that if HE grade increases by one, RB risk increases by 1.8 times, if HVPG increases by 1 mm, RB risk increases by 1.324 times, and if HVPG >14 mmHg, RB risk increases by 3.837 times.

HR <1 was significant for the independent variables: ascites grade and HVPG ≤14 mmHg (0.591 and 0.613, respectively). When HR <1, the effect of these factors was associated with increased survival time, i.e. a factor that reduces RB risk.

The quality of our chosen model of multivariate Cox proportional hazards regression is confirmed by estimating the maximum likelihood (log-likelihood or –2LL). In the SPSS program, this indicator of the model with predictors is compared with the indicator of the base model (without predictors) – Block 0. In our study in the base model (Block 0), the value of –2LL was 283.940, after introducing independent variables (predictors) into the model, –2LL decreased (237.457, Pearson's Chi-square = 57.385) at a significance level of 0.0001. This prevented the acceptance of the null hypothesis, which in fact means that the predictive ability of the multivariate Cox proportional hazards regression analysis model improved when independent predictors were included.

We constructed a correlation matrix to test the condition for multivariate Cox proportional hazards regression analysis (no linear relationship between independent variables, which creates redundancy in the model). The correlations found were very weak (–0.024 to 0.196), or weak (0.196 to 0.435) and of medium strength of expression (0.435 to 0.548), which does not negatively affect application of the model [20].

In multivariate analysis, we plotted the hazard ratio (HR) for different values of the categorical variable HVPG (≤14 mmHg; >14 mmHg) at LT waiting times of up to 60 weeks (Fig. 2).

As shown in Fig. 2, RB risk with HVPG >14 mmHg progressively increases in patients with LT waiting periods after 25 weeks of LT, while it is absent with HVPG ≤14 mmHg, reaching HR = 0.613 at 55 to 60 weeks of LT waiting time.

DISCUSSION

We showed that rebleeding occurred in 39 out of 51 patients (76.5%) who received first-line prophylactic therapy (EVL + NSBB) and were waiting for LT for up to 60 months. Rebleeding occurred early, before 6 weeks, in almost 25% of patients, and earliest, before 1 week, in 7.7% of cases.

We believe that the high frequency of RB in our study is down to several factors. First, as cirrhosis decompensation progresses, EVL has been shown to have no effect on HVPG [7–9]. Secondly, NSBB (propranolol + carvedilol) reduces HVPG in those patients who received prophylactic therapy (propranolol by 10.1% in 23.2% of cases, carvedilol by 18.6% in 27.7% of cases) [10]. Thirdly, administering NSBB without first determining the hemodynamic response to it at high HVPG levels, implies that nonresponders taking these medications may not respond to treatment [21, 22] and that EVL and NSBB combination therapy may be less effective [23].

Our research supported the findings of Ardevol et al. [10], who demonstrated that certain individuals develop early (up to 6-week) RB even while first-line therapy (EVL + NSBB) for up to 46 months provides satisfactory efficacy for secondary rebleeding prophylaxis. However, the incidence of RB within these periods in our study was nearly twice as high as the cited study. Perhaps this difference in the incidence of early recurrent hemorrhage between our study and the study by Ardevol et al. [10] is due to the larger sample of patients included (51 and 369 patients, respectively). This team of researchers

showed a high mortality in EVL + NSBB patients during RB prophylaxis, which is consistent with the study we have published earlier [23].

The high incidence of rebleeding in secondary prophylaxis is due to progression of cirrhosis with increasing disease duration (increasing MELD score, increasing HE and ascites grades, appearance of resistant ascites, bleeding) and increasing severity of clinically significant portal hypertension (CSPH) [18, 24–28].

Indicators such as hemoglobin, creatinine, MELD-Na and CTP scores, the number of consecutive variceal ligations, mean HVPG, and mean HVPG >14 mmHg differed between patients with and without RB. We listed these as likely risk factors for this adverse clinical outcome since the group of RB patients exhibited a substantial change in these parameters when compared to those without RB. According to foreign reports, variceal diameter [29], patient age, duration of the disease (cirrhosis), high CTP score and variceal size are risk factors for RB [3, 30].

When comparing patients with HVPG ≤14 mmHg and HVPG >14 mmHg, we showed that higher HVPG significantly increased the percentage of patients with RB who received first-line therapy prophylaxis. This is consistent with the results of many studies, showing that progressive increase in HVPG and lack of hemodynamic response to propranolol [33] are the most important factors in variceal hemorrhage [7–9, 28, 31] and their recurrences [32, 33].

We found that in both univariate and multivariate analyses, HE severity (grade), HVPG (cat.) and HVPG >14 mmHg were significant independent predictors of RB. Significant independent predictors (MELD-Na, CTP and creatinine level) in univariate analysis showed no significant effect on RB risk in multivariate analysis. It is known that CSPH signs start to appear at HVPG ≥10 mmHg, and that the progressive course of these symptoms correlates with progressive development of decompensation (larger variceal size with the risk of rupture, bleeding, ascites, HE) [28]. HVPG is a prognostic indicator for patients with cirrhosis [4, 7, 28] and is a commonly used predictor of ascites, HE, first bleeding episode and RB [11, 28].

Our findings somewhat agree with those of Yaru et al. [25], who found that HVPG size, HE, ascites, and CTP score are predictive of RB.

We found that RB risk increases 1.324 times when HVPG increases by 1 mm, and that RB risk increases 3.837 times when HVPG >14 mmHg, which is consistent with other reports. Through multivariate analyses using Cox logistic regression analysis, Zhao et al. [34] showed that for every 1 mm increase in HVPG, there is a 1.534-fold increase in the risk of early rebleeding within 6 weeks of the first bleeding episode with HVPG ≥20 mmHg [OR (odds ratio) = 1.534, 95% CI (CI): 1.062–2.216, p = 0.022)].

We have demonstrated that HVPG ≤14 mmHg and ascites severity have no prognostic value in predicting the risk of rebleeding because their respective hazard ratios in multivariate analysis using the Cox regression model is HR <1 (0.591 and 0.613, respectively). It is known that in survival analysis using Cox proportional hazards regression model, HR <1 indicates the impact of these factors, resulting in a decrease in the risk of an adverse event (RB in our instance). In support of these findings, we cite the study by Liu et al. [1] that showed that HVPG <15 mmHg is not a predictor of early RB occurring after the first bleeding episode in patients with ascites. Wu et al. [35] discovered that in patients with HVPG of 14 mmHg, rebleeding did not occur within a year following the initial bleeding episode, while in those with HVPG of 18 mmHg, rebleeding occurred in 23.6% of cases. Moreover, most rebleeding patients had HVPG exceeding 18 mmHg (51.3% vs. 31.0%, p = 0.021 compared to the group without bleeding.

CONCLUSION

Despite prophylaxis with first-line medication (EVL + NSBB) given after the first bleeding episode, RB occurs in up to 60 months of follow-up in 76.5% of patients who have been waiting for LT for several years due to lack of a donor organ. Of the patients, approximately 25% developed early RB within 6 weeks of the initial bleeding episode.

Progressive decompensated cirrhosis, development of CSPH manifested by ascites, HE, progressive increase in variceal size and variceal rupture, and variceal hemorrhage are the causes of RB with a prolonged wait for LT (about 60 months).

Changes in hemoglobin and creatinine levels, MELD-Na and CTP scores, the number of variceal ligations in a row, mean HVPG, and mean HVPG >14 mmHg are risk factors for RB. HVPG >14 mmHg is the primary predictor of RB, increasing the risk for a 1-mm change in gradient value by a factor of 3.837. Higher HE severity is the second independent predictor. RB risk increases with a 1.8-fold increase in HE severity.

Early TIPS procedure (preemptive TIPS) is required for a considerable number of patients with early RB before 6 weeks in order to lower the risk of recurrent bleeding and associated mortality.

The authors declare no conflict of interest.

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RELATIONSHIP BETWEEN ALLOGRAFT PERFUSION PREPARATION VARIATIONS AND RATE OF ARTERIAL AND BILIARY COMPLICATIONS IN ORTHOTOPIC LIVER TRANSPLANTATION

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Objective: to evaluate the possible influence of different graft perfusion preparation variations on the incidence of biliary and vascular complications of orthotopic liver transplantation (OLT). **Materials and methods.** Data on 287 full-size liver transplants from donors with brain death and beating heart were processed. There were 262 and 25 primary and repeat OLTs, respectively. Before completion of portal anastomosis formation and inclusion into systemic blood flow, the graft was perfused with hypo- (group 2) and isotonic (group 4) saline in order to minimize hemodynamic disorders. **Results.** There was a statistically significant difference between groups 2 and 4 in the development of late ($p = 0.04$) and cumulative biliary complications ($p = 0.01$). The presence of these complications and the perfusion type were found to be associated (Fisher's exact test = 0.02). There were no differences in incidence of thrombosis in the studied groups. **Conclusion.** The conducted analysis suggests that it is inexpedient to use hypothermic solutions when preparing a liver transplant for perfusion before introducing it into systemic circulation.

Keywords: liver transplantation, post-transplant biliary complications, allograft preparation for perfusion.

INTRODUCTION

Liver transplantation (LT) is the only effective treatment for progressive liver failure and life-threatening complications of portal hypertension [1–3]. The pressing concern in transplantation, notwithstanding the high standards of surgical procedure, is to lower the rate of complications that result in graft dysfunction and loss. Prolonged hypotension in the donor during conditioning, the recipient's severe state at the time of transplantation, and a significant amount of intraoperative blood loss can be an adverse background for complications [4, 5]. Cold and warm ischemia time and the severity of allograft steatosis are proven factors in the development of graft dysfunction and complications [6–8].

Various methods of physical, chemical, therapeutic and other effects on the harvested organ are used, potentially reducing the negative impact of conservation stages. In several reports, there are different data on preliminary preparation of the transplant before introducing it into the systemic circulation. The benefitsof both normothermic and hypothermic machine perfusion are described with the purpose of "functional" preservation of the allograft in donor after cardiac death and reduction in the intensity of ischemia-reperfusion syndrome in recipients [9–11]. It is worth considering the assertion made by some authors that retrograde perfusion, unlike the classical technique in LT, lowers the concentration of toxic metabolites in the allograft that build up during the preservation process. This reduces the risks of significant hemodynamic disorders when the graft is introduced into systemic circulation in contrast to the classical OLT technique and the severity of reperfusion syndrome [12, 13].

No less significant are the elimination of technical flaws at all stages of transplantation, and peculiarities of the postoperative period, where the graft's adequate arterial blood supply is crucial. In this regard, minimizing negative factors that influence the development of arterial and biliary complications is one of the primary goals to preserve its full function, which determines the quality of life and prognosis in the recipient [14, 15]. Prevention of viral hepatitis recurrences in a significant proportion of recipients also remains an urgent task [16].

Methods of statistical processing of information

Pearson's chi-square test was used to establish regularities between categorical variables, and Fisher's exact test was used when the expected frequency of a characteristic was less than 10. Parametric Student's t test and non-parametric Mann–Whitney U test were used to determine the differences between two quantifiable independent variables. Kruskal–Wallis analysis of variance was used to compare the medians of independent samples from three or more groups. The Kaplan–Meier life table method was used to estimate survival rates. The arithmetic mean and standard deviation were used as measures of descriptive statistics, and for heterogene-

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ous data or small samples, the median and interquartile range (IQR) of 25–75% (in square brackets) were used.

MATERIALS AND METHODS

General Information

During the period from June 1998 to April 2023, 287 full-size liver transplants from donors with brain death and beating heart were performed at the Russian Research Center of Radiology and Surgical Technologies ("Research Center"). Indications for OLT were irreversible liver disease leading to predicted death within 6–12 months, with no effect from ongoing conservative treatment. Viral hepatitis resulting in cirrhosis and cholestatic diseases were the most common causes of disease that led to OLT. When preparing the liver transplant, its color, edge, density, presence of focal changes and fibrosis were evaluated. If steatosis was suspected, the suitability of the organ for transplantation was reevaluated after cold perfusion with Custodiol solution (Custodiol HTK, Kohler, Germany). Subsequently, express morphologic examination of the biopsy specimen was required in 37 (12.9%) cases. In our Research Center, LT with macrovesicular steatosis of more than 50%, diagnosed by express biopsy, was not performed. During routine examination of native biopsy specimens according to the degree of severity of macrovesicular steatosis (1–4) of the graft, distribution was performed in the following order: grade 1 (1–10%), grade 2 (11–30%), grade 3 (31–50%), grade 4 (>50%).

Primary and repeat OLT was performed in 262 and 25 cases, respectively (retransplantation was performed twice in 3 recipients). There were 116 (44.3%) men and 146 (55.7%) women who underwent transplantation. Their age ranged from 18 to 64 years, the mean was 45.1 ± 11.3, and median 46.8 years [IQR 38.8–53.9 years].

Surgical stages of transplantation

The main surgical stages were standard, including isolation of liver from the ligamentous apparatus and adhesions, arterial, portal, and venous devascularization followed by hepatectomy. Caval reconstruction variants are shown in Table 1.

Portal reconstruction was performed by end-to-end anastomosis. Before the final completion of portal anastomosis, the allograft was perfused (flushed) through the portal vein in 400–500 mL of blood with further inclusion of the liver into the systemic circulation (group 1, n = 83).

The retrograde liver perfusion variation was not performed in our Research Center in any case.

Table 1

Reconstruction variants of caval anastomoses

Anastomosis variant	Number of observations	%
Classic technique	45	15.7
Cavocavostomy	65	22.6
Piggyback	177	61.7
Total	287	100

Graft perfusion preparation

Significant hemodynamic complications – persistent hypotension, arrhythmia and asystole – were frequently encountered at the stage of organ incorporation into systemic circulation. The cause of these conditions was the entry into the blood of excess amount of ionized potassium, contained both in the perfusate (custodiol) and leaving the cytosol of hepatocytes in the process of preservation. Also, the factor of rapid one-time blood loss during the final flushing of the organ with portal blood played a major role in the genesis of these hemodynamic disorders.

In order to minimize or prevent hemodynamic disorders before the final flushing of the allograft through the portal vein, we started to perfuse the graft with 500–1500 mL of saline cooled to 2–4 °C (group 2, n = 85), and then with addition of 10% albumin solution in order to approach the osmotic and oncotic blood parameters (group 3, n = 61). The justification for the use of hypothermic solutions was based on the published printed works about the possibility of prolonging the optimal preservation of graft and its energy resources [17–18]. Preliminary perfusion of the organ was terminated when the amount of ionized potassium in the fluid passing from the liver through the inferior vena cava (IVC) reached a physiological or low level. Saline perfusion into the portal vein lasted for 5–15 minutes, the pressure of the injected fluid did not exceed physiologic parameters (10–12 mmHg).

As experience was gained with the use of preliminary perfusion of organs with solutions of different temperatures, operating surgeons began to note the peculiarities of the donor liver condition. After washing with hypothermic solutions, at the stage of inclusion into systemic circulation, palpatory irregular density increase, mosaic coloration of the organ parenchyma, as well as decrease in volumetric velocity of blood flow through graft arteries after arterial reconstruction and start-up were observed. At the same time, the patency of graft arterial bed before and after anastomosis, and adequate outflow through hepatic veins were preserved. This could indicate a disruption in intraorgan microcirculation and an increase in peripheral vascular resistance. The abovementioned changes prompted us to abandon hypothermic perfusion in favor of isothermal perfusion.

We started perfusing the organ with saline at a temperature of 20–24 °C; the amount of fluid varied from 500 to 2000 mL. Following the commencement of portal blood flow in the transplant, 200–250 mL of blood were used for extra "flushing" of the liver through the IVC

subhepatic segment. This was followed by suturing of the "window" in the vein (group 4, n = 58) and inclusion of the transplanted organ into systemic circulation.

In order to evaluate the influence of the temperature factor and to correctly compare the perfusion preparation variations in the comparison groups, we used perfusion variation 2 (n = 85), where 500–1500 mL saline cooled to 2–4 °C was used, and perfusion variation 4 (n = 58), where saline with a 20–24 °C temperature was used, and the fluid volume varied from 500–2000 mL.

Groups 1 and 3 were not included in the comparative analysis due to different perfusion variations: no saline solution was injected into the group 1 graft, albumin was added in group 3.

Arterial reconstruction

Further, arterial reconstruction with standard anatomy was performed in most cases by forming an anastomosis with the donor's proper hepatic artery (PHA) (Table 2).

Arterial reconstruction with donor's proper hepatic artery (PHA) was performed in 70.4% (n = 202); in 57 of 202 cases, a common site with the donor's gastroduodenal artery was formed to match the diameters of the vessels being sutured. The second most frequent variant was anastomosis with the donor's common hepatic artery – 16.4% (n = 47). Separate anastomoses were used in 8.7% of cases (n = 25). This was due to the branching of the substitute right hepatic artery (RHA) of the donor organ by a separate trunk (from the superior mesenteric artery) or the presence of a significant accessory left hepatic artery (LHA). In these cases, reconstruction was most often performed by anastomosing with the recipient's RHA and LHA, respectively. Most arterial anastomoses were formed by continuous sutures (using the parachute technique) with Prolene 6/0 suture. A small number of anastomoses were formed by separate interrupted sutures using Prolene 7/0–8/0 suture. In 13 recipients (4.5%), the graft arteries were sutured directly to the aorta using a vascular graft. In 9 out of 13 cases, this technique was used in liver retransplantation due to severe scar adhesions in the subhepatic space.

Table 2

Reconstruction variants of arterial anastomosis

Reconstruction variant	n	%
PHA (d) – vessels (p)	202	70.4
CHA (d) – vessels (p)	47	16.4
Split anastomosis	25	8.7
Aortic anastomosis	13	4.5
Total	287	100

Note: PHA, proper hepatic artery; CHA, common hepatic artery.

Table 3

Biliary reconstruction variants

Biliary reconstruction variant	n	%
BBA with external drainage	161	56.1
BBA on lost drainage	39	13.6
BBA without drainage	53	18.5
HPA	28	9.7
Cholangiostomy	4	1.4
Other	2	0.7
Total	287	100

Note: BBA, biliobiliary anastomosis; HPA, hepatic portoenteroanastomosis.

Biliary reconstruction

Biliobiliary anastomosis was formed in the vast majority of transplantations, including repeat transplants, when the recipient's bile duct was identified (Table 3). At the beginning of the practice, biliodigestive anastomosis on the loop of small intestine disconnected by Roux was performed in recipients with primary sclerosing cholangitis and at retransplantations. However, with our own experience, we concluded that primary biliodigestive anastomosis is applicable only in cases of "absence" of normal anatomy of the recipient's common bile duct, patency of its lumen after revision of hepatoduodenal ligament elements, less often in case of repeat OLT.

When transecting the graft bile duct, the degree of adequacy of blood supply at its incision was assessed; if necessary the duct was dissected proximally. When forming a biliobiliary anastomosis, we used absorbable suture material (PDS 6/0, less often 5/0), a separate interrupted suture. If there was obvious mismatch between the diameters of the ducts to be sutured (small diameter of the donor part), we resorted to combining the hepatic and cystic ducts into one site. Different drainage variants were resorted to in the event that reconstruction proved problematic or the course was expected to be complicated (presence of cholangitis, pancreatitis, sludge syndrome). Since 2019, almost no external drainage has been done.

Hypothesis and justification for its possible causes

The reason for the study was the differences in the incidence of postoperative biliary complications for different variations of transplant perfusion preparation (Table 4).

To analyze possible factors that are directly or indirectly influencing biliary complications, the following were evaluated:

1. Cold and warm time,
2. Duration of anhepatic phase,
3. Arterial reconstruction time (before its start),
4. Intraoperative blood loss (estimated by the volume of exchange transfusion),

5. Severity of patient's condition according to the MELD score at the time of transplantation (data before 2007 were considered retrospectively).

Evaluating the influence of the severity of allograft macrovesicular steatosis on the development of post-transplant complications, a study of biopsies of the "native" liver was conducted in 141 (98.6%) of 143 recipients (perfusion variations 2 and 4). No biopsy was performed in 2 cases.

The correlation of problems of arterial blood supply to the graft with the development of postoperative biliary complications was analyzed. Taking into account the leading role of arterial insufficiency in biliary complications, we also carried out the statistical processing of preservation parameters and intraoperative indicators of the problems of arterial blood supply of the graft. We took into account all the facts of its surgical and x-ray endovascular correction performed intraoperatively, as well as in the early and late periods after liver transplantation.

RESULTS

When analyzing the incidence of biliary complications in the comparison groups with perfusion variations 2 and 4, the following results were obtained (Table 5).

There was a statistically significant difference between groups 2 and 4 in the development of late* (p = 0.04) and all* biliary complications (p = 0.01).

In terms of severity of graft steatosis, the perfusion variations 2 and 4 groups, who subsequently had early biliary complications, were comparable (Cochran–Armitage test for trend p = 0.130).

In recipients (perfusion variations 2 and 4) with late biliary complications, the following results were obtained (Cochran–Armitage test for trend, p = 0.026), Table 6.

Thus, the majority of allografts of both study groups had grade 1 steatosis (1–10%), and did not statistically differ from each other (p = 0.063) in the development

Table 4

Frequency of biliary complications

Perfusion variation	Number of early complications (n/%)	Number of late complications	Total
1 (n = 83)	2 (2.4%)	7 (8.4%)	9 (10.8%)
2 (n = 85)	15 (17.6%)	14* (16.5%)	29* (34.1%)
3 (n = 61)	5 (8.2%)	8 (13.1%)	13 (21.3%)
4 (n = 58)	6 (10.3%)	3* (5.2%)	9* (15.5%)
Total (n = 287)	28 (9.8%)	32 (11.1%)	60 (20.9%)

Note: *, p < 0.05.

Table 5

Frequency of biliary complications in groups 2 and 4

Perfusion variation	Number of early complications (n/%)	Number of late complications	Total
2 (n = 85)	15 (17.6%)	14* (16.5%)	29* (34.1%)
4 (n = 58)	6 (10.3%)	3* (5.2%)	9* (15.5%)
p (CI <95%)	0.23	0.4	0.01
Total (n = 143)	21 (14.7%)	17 (11.9%)	38 (26.6%)

Note: *, p < 0.05.

Table 6

Distribution of recipients by degree of macrovesicular steatosis

Degree of steatosis	Complications (perfusion variation 2)		P (CI <95%)
	No	Yes	
1 (1–10%)	52 (61.2%)	13 (15.3%)	0.811
2 (11–30%)	1 (1.2%)	1 (1.2%)	0.293
3 (31–50%)	1 (1.2%)	0	0.614
4 (>50%)	1 (1.2%)	0	0.614
Degree of steatosis	Complications (perfusion variation 4)		P (CI <95%)
	No	Yes	
1 (1–10%)	40 (69%)	3 (5.2%)	0.446
2 (11–30%)	7 (12.1%)	0	0.481
3 (31–50%)	1 (1.7%)	0	0.803

of complications. It is worth noting the relatively small sample of steatosis grade 2 and higher in both groups. However, its severity with and without complications in recipients was not significantly different in both study groups.

The indicators of the studied factors of late complications (Table 7), in their absence (Table 8), as well as generalized data (Table 9) are presented below.

Table 7 shows that there is a two-fold difference between median duration of arterial reconstruction and of

Table 7

Analyzed indicators in recipients with late biliary complications

Parameters/ perfusion		Medians				
	CI (min)	WI (min)	AHP (min)	Art.Rec (min)	MELD	ET (mL)
2	355 [253.8–426.3]	50 [38.8–60]	82.5 [65–98.8]	32.5 [25–48.80]	17 [16–20]	846.5 [401–1631.8]
4	275 [275–282.5]	50 [40–70]	115 [80–120]	62.5 [40–73.8]	17 [12–18]	1660 [828–2387]
p (CI <95%)	0.432	0.676	0.768	0.264	0.953	0.3

Test Statistics[a]

	ET (mL)	MELD	CI (min)	WI (min)	AHP (min)	Art.Rec (min)
Mann–Whitney U	12.000	20.000	14.000	17.000	18.000	5.000
Wilcoxon W	117.000	26.000	20.000	122.000	123.000	83.000
Z	–1.134	–0.129	–0.885	–0.509	–0.379	–1.287
Asymp. Sig. (2-tailed)	0.257	0.897	0.376	0.611	0.705	0.198
Exact Sig. [2*(1-tailed Sig.)]	0.3	0.953	0.432	0.676	0.768	0.264

Note: WI, warm ischemia; CI, cold ischemia; AHP, anhepatic phase; Art.Rec, arterial reconstruction duration; MELD, Model of End-stage Liver Disease; ET, exchange transfusion; *, $p < 0.05$.

Table 8

Analyzed indicators in recipients without biliary complications

Parameters/ perfusion		Medians				
	CI (min)	WI (min)	AHP (min)	Art.Rec (min)	MELD	ET (mL)
2	330 [281.3–402.5]	45 [40–55]	75 [65–93.8]	30 [23.8–42.5]	17.5 [13–21]	842 [406–1973]
4	325 [262.5–397.5]	60 [45–65]	85 [70–105]	40 [30–53.8]	19 [15.5–22]	1817 [817.3–2400]
p (CI <95%)	0.321	0.001*	0.017*	0.035*	0.1	0.003*

Test Statistics[a]

	ET (mL)	MELD	CI (min)	WI (min)	AHP (min)	Art.Rec (min)
Mann–Whitney U	896.000	1121.500	1217.500	864.000	1003.000	749.500
Wilcoxon W	2492.000	2717.500	2442.500	2460.000	2599.000	1652.500
Z	–2.921	–1.614	–0.993	–3.284	–2.377	–2.106
Asymp. Sig. (2-tailed)	0.003*	0.107	0.321	0.001*	0.017*	0.035*

Note: WI, warm ischemia; CI, cold ischemia; AHP, anhepatic phase; Art.Rec, arterial reconstruction duration; MELD, Model of End-stage Liver Disease; ET, exchange transfusion; *, $p < 0.05$.

Table 9

Analyzed parameters of all recipients with perfusion variations 2 and 4

Parameters/ perfusion		Medians				
	CI (min)	WI (min)	AHP (min)	Art.Rec (min)	MELD	ET (mL)
2	330 [270–410]	45 [40–55]	75 [62.5–92.5]	30 [25–55]	17 [14–20.5]	931 [431–1742.5]
4	295 [263.8–386.25]	55 [45–65]	85 [70–105]	40 [25–50]	18.5 [17.8–23]	1759 [765–2387]
p (CI <95%)	0.199	0.001*	0.006*	0.207	0.1	0.004*

Test Statistics[a]

	ET (mL)	MELD	CI (min)	WI (min)	AHP (min)	Art.Rec (min)
Mann–Whitney U	1738.500	2067.000	2153.000	1671.500	1797.500	1654.500
Wilcoxon W	5393.500	5722.000	3864.000	5326.500	5452.500	4000.500
Z	–2.847	–1.641	–1.283	–3.282	–2.752	–1.261
Asymp. Sig. (2-tailed)	0.004*	0.101	0.199	0.001*	0.006*	0.207

Note: *, $p < 0.05$.

exchange transfusion. Nevertheless, in all parameters examined, there was no statistically significant difference between the groups with perfusion variations 2 and 4.

When analyzing the parameters (Table 8), there was a statistically significant difference in warm ischemia time, anhepatic phase duration, arterial reconstruction and volume of exchange transfusion (more in group 4).

In all patients from the analyzed groups (2 and 4), there was a statistically significant difference in warm ischemia time, anhepatic phase duration, and exchange transfusion volume, which prevailed in group 4.

In order to determine the influence of the transplant perfusion preparation variations (groups 2 and 4), the following statistical processing was performed (Table 10).

Estimating the odds ratios and relative risk, the chances of complications with variation 2 perfusion are 2.819 times higher than with variation 4.

Regression analysis models were used to identify the possible impact of the analyzed parameters (as risk factors) on the incidence of biliary complications. Preliminary testing showed that distributions in the sample of features, except for MELD, were significantly different

Table 10

Analysis of correlation of biliary complications with perfusion variations

Complications * Perfusion Cross Tabulation (a)

			Perfusion		Total
			2	4	
Complications	No	Count	56_a	49_b	105
		% within	65.9%	84.5%	73.4%
	Early	Count	15_a	6_a	21
		% within	17.6%	10.3%	14.7%
	Late	Count	14_a	3_b	17
		% within	16.5%	5.2%	11.9%
Total		Count	85	58	143
		% within	100.0%	100.0%	100.0%

The results demonstrate that there were no complications in 65.9% cases of variation 2 perfusion and in 84.5% cases of variation 4 perfusion. Late complications were more frequent in perfusion 2 (16.5%) than in perfusion 4 (5.2%), $p < 0.05$.

Complications Perfusion Cross Tabulation (b)

			Perfusion		Total
			2	4	
Complications	No	Count	56_a	49_b	105
		% within	65.9%	84.5%	73.4%
	Yes	Count	29_a	9_b	38
		% within	34.1%	15.5%	26.6%
Total		Count	85	58	143
		% within	100.0%	100.0%	100.0%

According to general processed data for groups 2 and 4, it was noted that complications appeared significantly more frequently in variation 2 perfusion (34.1%) than in variation 4 (15.5%).

Chi-Square calculation for the table by five methods (c)

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	6.113[a]	1	0.013		
Continuity Correction	5.197	1	0.023		
Likelihood Ratio	6.414	1	0.011		
Fisher's Exact Test				0.020	0.010
Linear-by-Linear Association	6.070	1	0.014		
N of Valid Cases	143				

Fisher's exact test ($p = 0.02$) is presented for the processed data.

Risk Estimate (d)

	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for Perfusion (2 / 4)	2.819	1.217	6.534
For cohort Compl_b = 1	2.199	1.126	4.293
For cohort Compl_b = 2	0.780	0.646	0.942
N of Valid Cases	143		

from normal, and statistical significance at preliminary testing was insignificant ($p > 0.05$).

Analysis of transplant arterial insufficiency

Proceeding from the proven association between transplant arterial insufficiency and biliary complications, an analysis of factors potentially influencing the development of intraoperative, early and late thrombosis and graft arterial insufficiency was done (generally – transplant arterial insufficiency, Table 11).

Analyzing the data in Table 11, we obtained a significant difference in the frequency of intra- and postoperative complications. However, there were no significant difference when comparing cumulative complications between groups 2 and 4 ($p = 0.96$).

Direct ultrasound flowmetry and duplex scanning of liver vessels in all recipients were used to assess the arterial volumetric blood flow velocity.

Intraoperative thrombosis of the hepatic artery trunk or its lobular branches, which required repeated reconstruction, was observed in 4 cases (2.8%), of which 3 recipients underwent separate lobular arterial anastomosis. Early and late postoperative thrombosis developed in 5 (3.5%) and 2 (1.4%) cases, respectively. In 6 (4.2%) cases, arterial blood supply in the graft was restored by means of X-ray endovascular techniques, only in one case was open thrombectomy performed (Table 12).

As follows from Table 12, there was no significant difference in the incidence of thrombosis in perfusion groups 2 and 4.

After conducting a statistical analysis of the factors under investigation, we found that groups 2 and 4 differed significantly in terms of volume of exchange transfusion required for all cases of thrombosis and the severity of the condition (MELD) in intraoperative thrombosis cases (Table 13).

In 3 (3.5%) cases from group 2, intraoperative correction of graft arterial blood supply was performed due to anastomosis deformity, kinking or doubts about arterial patency. This situation was more often observed when anastomosis was formed separately with the lobar arteries. Anastomoses were revised using a Fogarty catheter, the vascular bed was heparinized, and if there were no improvements, repeated arterial reconstruction was carried out. In one case, arterial inflow was corrected endovascularly.

Transplant arterial insufficiency was assumed on the basis of a decrease in volumetric velocity of blood flow through the hepatic artery to less than 100 mL/min (according to intraoperative flowmetry), provided that there were no palpatory and visual signs of anastomotic

Table 11

Frequency of liver transplant arterial insufficiency in different periods

Group (n)	Intraoperative (n/%)	Postoperative (n/%)		Total (n/%)
		Early	Late	
2 (n = 85)	15/17.6	5/5.9	9/10.6	29/34.1
4 (n = 58)	1/1.7	18/31	1/1.7	20/34.4
p (CI 95%)	0.003*	0.001*	0.04*	0.96
Total: 143 OLT	16/11.2	23/16.1	10/7	49/34.3

Note: *, $p < 0.05$.

Table 12

Frequency of arterial thrombosis in OLT

Group (n)	Intraoperative thrombosis (n/%)	Postoperative thrombosis (n/%)		Total (n/%)
		Early	Late	
2 (n = 85)	3/3.5	4/4.7	2/2.4	9/10.6
4 (n = 58)	1/1.7	1/1.7	–	2/3.4
p (CI 95%)	0.52	0.34	0.24	0.12
Total: 143 OLT	4/2.8	5/3.5	2/1.4	11/7.7

Table 13

Differences in the studied parameters

Parameters	ET medians (mL, all thromboses)	MELD medians (intraoperative thrombosis)
Group 2	1193 [566.5–2202]	15 [11–19]
Group 4	1759 [800–2395]	18.5 [16–22.5]
p (CI 95%)	<0.05	<0.05

Table 14

Correlation between vascular and biliary complications in early and late postoperative period

Perfusion variation	Arterial insufficiency / number of early complications (n (%))	Arterial insufficiency / number of late complications (n (%))	Total
1 (n = 83)	0/2	3/7 (42.8%)	3/7 (42.8%)
2 (n = 85)	13/15 (86.7%)	7/14 (50%)	20/29 (69%)
3 (n = 61)	4/5 (80%)	3/8 (37.5%)	7/13 (53.8%)
4 (n = 58)	6/6 (100%)	1/3 (33.3%)	7/9 (77.8%)
Total (n = 287)/60	23/28 (82.1%)	14/32 (43.8%)	37/60 (61.7%)

obstruction, and blood pressure level was not lower than 100 mmHg.

In order to correct the volumetric velocity of blood flow through the hepatic artery in 8 cases (9.4%) from group 2, we skeletonized the main arteries supplying the liver, including ligation of the gastroduodenal and splenic arteries (in one case, the left gastric artery was ligated). This resulted in a significant increase in arterial contribution to hepatic blood flow. Overall, hepatic artery skeletonization to increase volumetric blood flow velocity was performed in 31 cases. However, in the early and late postoperative periods, these patients developed biliary complications in 3 and 1 cases, respectively.

When analyzing the possible causes of early transplant arterial insufficiency, we found a statistically significant difference in warm ischemia time. In groups 2 and 4, the medians were 45 [40–60] and 55 [45–65] minutes, respectively ($p < 0.05$). There was a difference in exchange transfusion volume – 750 [401–1618] vs 1718 [2398–6497] mL in groups 2 and 4, respectively ($p < 0.05$).

When analyzing the correlation between pre-existing transplant arterial insufficiency and biliary complications, the following data were obtained for different periods (Table 14).

According to Table 14, pre-existing transplant arterial insufficiency was highly likely to be the cause of early biliary complications.

DISCUSSION

It is known that shortening the preservation stage prevents graft dysfunction in OLT and reduces the risk of early and late postoperative complications [19]. The desire to maximize preservation of allograft from expanded criteria donors (and donors after cardiac death) divided the researchers into two camps, explaining the advantages of both hypothermic and normothermic machine perfusion. According to reports, the hypothermic option allows prolonging allograft safety in the process of preservation, while the use of the normothermic method is recommended when using a suboptimal graft [20–23].

The methods of perfusion with hypothermic solutions used suggest a reduction in expenditure of liver energy reserves in the allograft preservation process; nonetheless, considering the other resulting effects, they remain a matter of debate. Summaries from randomized controlled trials in published works are scarce. To lessen the adverse effects of warm ischemia, we prepared the graft using cooled solutions due to our lack of experience with retrograde and machine perfusion. Prevention of acute cardiovascular diseases due to ionized potassium inflow into systemic circulation and one-time blood loss during graft flushing with portal blood was also considered important.

Perfusion with hypothermic solutions against the background of warm ischemia may aggravate endothelial cell damage (most significantly in sinusoidal veins). This could compromise microcirculation within the graft. We did not use retrograde caval reperfusion. The rationale for this technique is provided in published works – when the graft is introduced into systemic circulation, there is no rapid entry of excess ionized potassium or anaerobic metabolic products into the graft. Together with a decrease in the concentration of vasoactive substances, this reduces the severity of reperfusion syndrome manifestations [24]. However, the liver transplant reperfusion experience across various centers prevents us from giving preference to a clear-cut technique for its implementation [25].

As the number of transplantations increased, we gained more experience in vascular and biliary complications in OLT, which became the most frequent and significant causes of graft dysfunction and graft loss in the postoperative period [26]. Analysis of biliary complications seemed to be the easiest and most informative to process. A significant difference was obtained in the development of late (16.5 *vs.* 5.2%) and cumulative (34.1 *vs.* 15.5%) biliary complications in groups 2 and 4 ($p < 0.05$). This necessitated a comparison of preservation parameters, intraoperative data and severity of condition (MELD) in the studied groups.

In recipients of the analyzed groups with complications, there was no statistically significant difference in the analyzed characteristics. In cases without biliary complications, there was a significant excess in warm ischemia time, anhepatic phase, arterial reconstruction and exchange transfusion volume in group 4 ($p < 0.05$).

In the groups (2 and 4), there were significant differences ($p < 0.05$) in warm ischemia time, anhepatic phase, and exchange transfusion volume. Moreover, despite

the unfavorable background in group 4, complications developed less frequently.

The analysis established the comparability of groups with different perfusion variations in terms of severity of steatosis. A possible drawback in information processing is the small sample size for steatosis of grade 2 or more. But this is retrospective.

Fisher's exact test was used to determine the possible correlation between biliary complications and perfusion variations (2 and 4); the result was $p = 0.02$. It cannot always be calculated, but if it exists, then you need to focus on it. Thus, there is a relationship between complications and perfusion type. In our study, perfusion with hypothermic solutions against the background of warm ischemia could aggravate endothelial cell injury (most significantly in the sinusoidal veins), and as a result, lead to impaired microcirculation in the graft.

When calculating the odds ratio and relative risk, complications with variation 2 perfusion are 2.819 times higher than with variation 4; variation 2 is 2.819 times higher than variation 4. There is also a confidence interval to this odds ratio. For the cohort of patients with biliary complications, the odds of belonging to perfusion 2 are 2.199 times higher than perfusion 4. Stated differently, perfusion 2 has a 2.199-fold higher risk of getting complications than perfusion 4 ($p < 0.05$). With the regression analysis models used, we were unable to determine how the examined parameters affected the frequency of biliary complications. Similarity between groups 2 and 4 in terms of preservation and steatosis severity, as well as abnormal distribution in the samples, could be one reason for this.

Considering the results obtained in recipients with transplant arterial insufficiency, intraoperative and late disorders were predominant in group 2 ($p < 0.05$), while early disorders predominated in group 4 ($p < 0.05$).

There was a significant predominance of medians in group 4 with regard to exchange transfusion volume for all thromboses, and severity (MELD) of intraoperative thrombosis, although no difference in the incidence of thrombosis was found among the studied groups.

In the early postoperative period, after routine doppler ultrasound of the graft, splenic artery steal syndrome was ruled out in cases of suspected hepatic ischemia (without signs of impaired mechanical patency), low peak systolic velocity and diastolic component, high resistance (RI >0.80), abnormal dynamics of blood biochemical parameters (bilirubin, transaminases, INR). The diameter of the splenic artery exceeded the hepatic artery 1.5 times or more; there was severe splenomegaly and portal hyperperfusion (Fig. 1–3). This was considered an indication for direct angiography, which confirmed low volumetric blood flow. In our observations, the median resistive index in these patients with variation 4 perfusion was 0.86 [0.835–0.955].

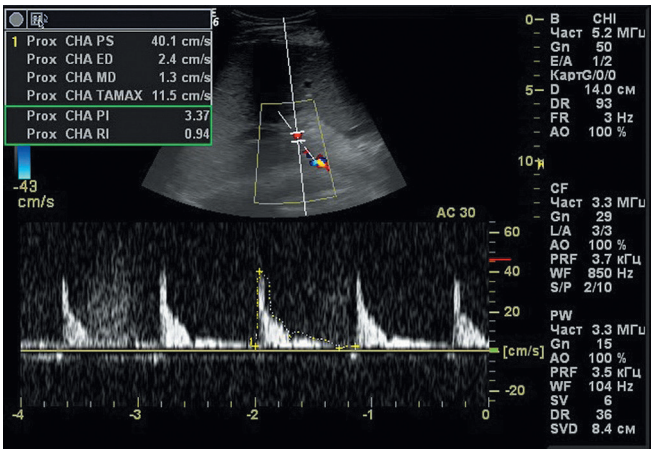

Fig. 1. Low-velocity blood flow in hepatic artery with high intravascular resistance (RI = 0.94)

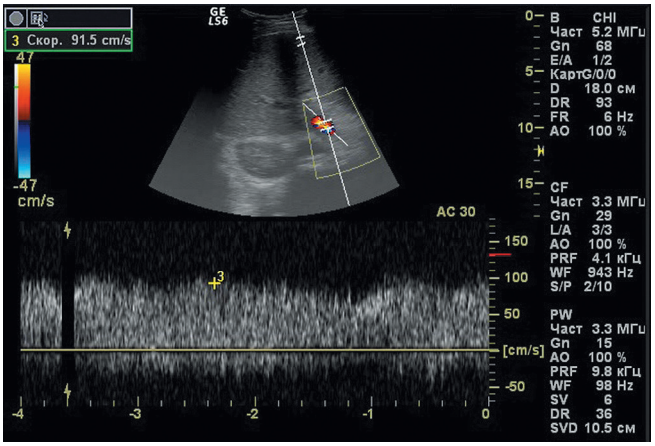

Fig. 2. Accelerated blood flow in the portal vein and hyperperfusion

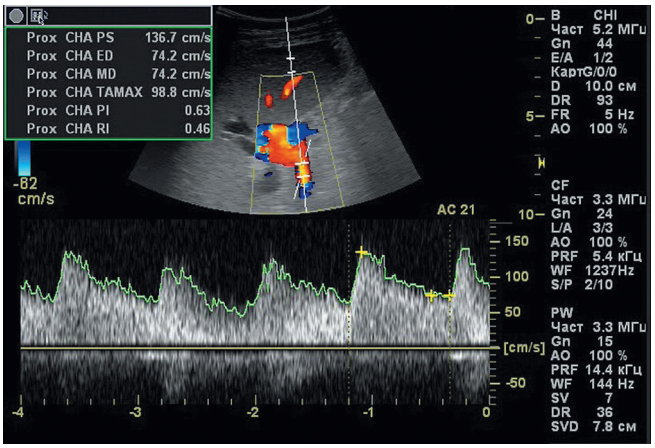

Fig. 3. Hyperdynamic blood flow in splenic artery

The syndrome of splenic artery stealing was confirmed by angiography. Blood supply of the graft was improved by splenic artery embolization (Fig. 4, 5).

In this regard, active management tactics for recipients with suspected transplant arterial insufficiency in

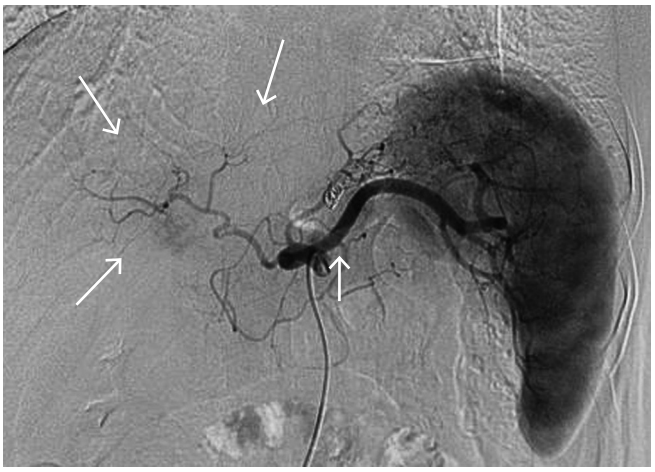

Fig. 4. Celiacography. Diameter of the splenic artery is 11 mm and hepatic artery is 4 mm. Impoverishment of hepatic arterial architectonics at the segmental level (arrows)

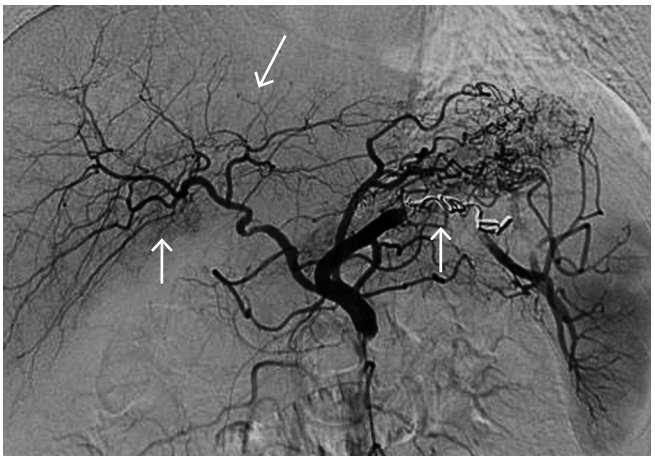

Fig. 5. Redistribution embolization of the splenic artery trunk. Restoration of hepatic arterial architectonics at the segmental level (arrows)

group 4 in the early postoperative period can explain the high rates presented in Table 11. It is possible that this subsequently resulted in reduced incidence of biliary complications after OLT.

Assessing the relationship between the incidence of early biliary complications and pre-existing transplant arterial insufficiency in the graft, in most cases there was a correlation with arterial insufficiency.

CONCLUSION

Group 4 exhibited a significantly longer warm ischemia and anhepatic phase, as well as a higher exchange transfusion volume. However, this unfavorable background did not lead to increased number of biliary complications in comparison with the group where hypothermic graft perfusion was performed. In terms of severity of graft steatosis, perfusion regimens 2 and 4, which subsequently had biliary complications, were comparable. Statistical analysis revealed a correlation between incidence of biliary complications and specific perfusion type. These findings suggest that it is inappropriate to use hypothermic solutions in perfusion preparation of a liver transplant before introducing it into systemic circulation.

The authors declare no conflict of interest.

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MUCORMYCOSIS IN SOLID ORGAN TRANSPLANT RECIPIENTS (CLINICAL CASES AND LITERATURE REVIEW)

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Mucormycosis is a severe mycotic infection with high mortality among immunocompromised patients. Its incidence in solid organ transplant recipients is 2–8% of all invasive fungal infections. In most cases, it occurs in the late posttransplant period. Risk factors in this patient cohort are graft-versus-host disease (GvHD) and use of immunosuppressive drugs. The article describes clinical cases of mucormycosis and analysis of literature data on the problem of invasive mucormycosis in solid organ transplant recipients. It also reviews the main methods of diagnosis and treatment of the disease according to international guidelines.

Keywords: antimycotic therapy, liposomal amphotericin B, isavuconazole, mucormycosis, graft-versus-host disease, GvHD, transplantation, internal organ transplantation, liver transplantation, kidney transplantation.

INTRODUCTION

Mucormycosis is a severe infection that predominantly occurs in immunocompromised patients. Solid organ transplant recipients are one of the risk groups for this nosology. Internal organ transplantation (IOT) is a life-saving intervention. The number of solid organ transplants in Russia is increasing annually. In 2022, 2,555 organ transplants (1,562 kidney, 659 liver, 310 heart) were performed [1], and by December 2023, 2,906 (1728 kidney, 789 liver, 371 heart, 17 lung, 1 heart-lung) [2]. Increased IOTs has also been reported by numerous international publications [3]. This patient cohort has a high risk of infectious fungal complications such as invasive candidiasis, aspergillosis, cryptococcosis, pneumocystosis and mucormycosis [4].

Mucormycetes are widespread. The main causative agents of mucormycosis belong to the order Mucorales (*Rhizopus spp., Mucor spp., Lichtheimia spp.* (formerly *Absidia spp.*), *Rhizomucor spp.* and *Apophysomyces spp.*). Although the disease is considered a rare infection [5, 6], there was a rise in incidence worldwide during the COVID-19 pandemic [7]. The most common clinical forms of the disease are rhinocerebral mucormycosis (20–60%) and pulmonary mucormycosis (20–70%). The infection spreads rapidly, involving nearby organs – about 50% of all cases [6]. Mortality averages 28% and depends on the clinical form of the disease (from 10% for sinusitis and skin lesions to 90% for the central nervous system (CNS) lesions and dissemination) [6–10]. International and Russian recommendations have demonstrated the need for primary antimycotic prophylaxis in patients at high risk of developing the disease, as well as early antimycotic therapy and surgical treatment [11, 12].

Objective: to analyze published data in order to determine the main risk factors, etiology, clinical manifestations and treatment outcomes of invasive mucormycosis in IOT recipients.

MATERIALS AND METHODS

The article presents clinical cases of disseminated mycotic rhinosinusitis, involving the orbital and CNS tissues. The EORTC/MSG criteria, 2020 [13] were used to make a diagnosis of invasive fungal disease.

The authors analyzed publications on mucormycosis in patients after organ transplantation. PubMed (as of December 2023), ClinicalKey (as of December 2023), and e-library (as of December 2023) were used. The following keywords were used in the information search: antifungal therapy, mucormycosis, graft-versus-host disease, GvHD, transplantation, internal organ transplantation, liver transplantation, kidney transplantation.

CLINICAL CASE #1

A boy S., 10 years old, was admitted to the Children's Republican Clinical Hospital, Kazan on July 21, 2021 with complaints of lack of urine, abdominal pain, noisy breathing, impaired consciousness.

Past medical history: *The child fell ill acutely, his condition deteriorated progressively on July 16, 2021 –*

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his body temperature rose to 38.5 °C, stool increased to five times a day, with an episode of acholia. He received ibuprofen, which reduced his body temperature to 37.8 °C. A day later, he started vomiting and experiencing pain in the right hypochondrium. At stoma opening, 20 ml of bile was flowing out (cholangiostomy performed in 2021). Immunosuppressive therapy and accompanying therapy (orthotopic transplantation of a liver fragment from a related donor in 2020) were reduced. Within three more subsequent days, the patient's condition progressively worsened – the child was lethargic, motor activity and appetite decreased, he complained of nausea, experienced low urine output (up to 70–100 mL of urine), and developed dyspnea. The child was urgently hospitalized.

***Life history:** A child after two pregnancies without pathology, 1 birth (1 miscarriage). Discharged from the maternity hospital with physiological jaundice. Jaundice syndrome increased from four months of life. Viral hepatitis B and C were ruled out, the clinical picture was considered as cytomegalovirus (CMV) infection; a course of antiviral therapy was administered. The course of jaundice syndrome in the boy was wavy. The jaundice syndrome intensified against the background of intercurrent diseases. Acholia, vomiting and regurgitation occurred periodically. At the age of 7 months, the boy started experiencing severe skin itching. Low weight and height gain, and delayed motor development attracted attention. A liver biopsy was performed in 2013 at the age of two years. Histological conclusion: Alagille syndrome. Congenital intrahepatic bile duct hypoplasia (Alagille syndrome) with cholestasis syndrome was diagnosed. Conservative therapy was administered: ursodeoxycholic acid, fat-soluble vitamins, nutrition therapy. The disease gradually progressed and in 2019 (8 years of life), a follow-up examination detected cirrhosis.*

On August 5, 2020, he underwent hepatectomy with preservation of inferior vena cava and orthotopic liver fragment transplantation from a living related donor (aunt); on September 24, 2020, his hepatic artery was stented and splenic artery embolized; the hepatic artery was re-stented on October 27, 2020. He received immunosuppressive therapy with calcineurin inhibitors (tacrolimus 2 mg) and glucocorticoids (methylprednisolone 6 mg). Liver graft dysfunction signs were detected repeatedly in 2021. He received complex antibacterial, immunosuppressive, antiplatelet, diuretic, choleretic and replacement therapy. On February 5, 2021, a cholangiostomy was installed.

***Physical examination upon admission to the hospital:** his condition was extremely severe due to multiple organ dysfunction syndrome (respiratory, cardiovascular and acute renal failure) and systemic inflammatory response syndrome (up to 38.5 °C. fever), neurological symptoms (stunning) and metabolic disorders. On auscultation, heart tones were muffled, rhythmic, heart rate was 130 per minute, and he had fast breathing (40 per minute). Saturation was 86–88%.*

***Laboratory tests.** Full blood count test: white blood cells 6.9 × 10^9/L, neutrophils 4.0 × 10^9/L, and lymphocytes 1.3 × 10^9/L. Chemistry panel: urea 34 mmol/L, creatinine 350 μmol/L, hyponatremia 129 mmol/L, C-reactive protein 10 mg/dL. Blood acid-base balance: pH 6.1–6.9, BE 24 mmol/L. Lung ultrasound: areas of reduced lung airiness – multiple B-lines (interstitial edema), no free fluid in the pleural cavities. Examination of throat and nasal swabs by PCR for SARS-CoV-2 RNA tested positive.*

Diagnosis: severe COVID-19 coronavirus infection, acute kidney injury grade 3 (KDIGO, 2012), oligoanuria; congenital hypoplasia of intrahepatic bile ducts (Alagille syndrome) with cholestasis syndrome; liver cirrhosis; orthotopic liver transplantation from related donor on August 5, 2020; hepatic artery stenting on April 29, 2020, hepatic artery re-stenting on October 27, 2020; cholangiostomy (February 5, 2021).

The following treatment was carried out: mechanical ventilation, antibacterial (cefepime + sulbactam, metronidazole, meropenem), anticoagulant and antifungal (caspofungin 70 → 50 mg/m^2) therapy, intravenous immunoglobulin 0.4 g/kg/day, pulse therapy with glucocorticoids (metipred until July 27), then dexamethasone 4 mg/day, tacrolimus was discontinued.

On July 22, 2021, the boy had a nosebleed, anterior and posterior nasal swab was performed. The child's condition worsened – two days after being hospitalized, viral (COVID-19) pneumonia was diagnosed. On July 27, 2021, signs of thrombotic microangiopathy (TMA) were revealed, respiratory failure, hemic hypoxia (severe anemia) increased. 20 mL/kg of fresh frozen plasma (FFP) and leukoreduced red blood cells were transfused. Signs of hepatic failure appeared, and therefore the dose of antibacterial drugs and caspofungin was reduced.

On day 12 of hospitalization (August 2, 2021), there were complaints of swelling and pain in the left eye. OS: eyelids were swollen, slightly hyperemic, edema mainly of the lower eyelid. In the area of the medial-inferior corner of the orbit, a voluminous dense mass, painful on palpation, was detected (Fig. 1). The conjunctiva was slightly hyperemic, swollen, mainly around the lower eyelid. Eye movements were full and painless. Deviation 0.

Antibacterial therapy was continued. Four days later, a lower eyelid abscess developed. The abscess was opened with subsequent drainage. A scanty pale-yellow discharge was isolated from the abscess cavity. Surrounding tissues were imbibed with pus. The operation went without complications. The drainage from the wound was removed on August 9, 2021. There was no discharge from the wound.

On August 10, 2021, there were complaints of impaired nasal breathing on the left side. A dense mass

was detected in the left nasal cavity, fused with nasal mucosa; a defect in the soft tissues of the hard palate was detected, the palatine bone was exposed.

Computed tomography (CT) scan of paranasal sinuses (dated August 11, 2021): foci of destruction in the medial walls of the orbits, in the bones of the nasal cavity, hard palate in the alveolar processes of the maxillary bones in the plates of the pterygoid process on the left upper wall of the ethmoidal labyrinth (Fig. 2, a). Brain CT scan (dated August 11, 2021): rounded foci in the frontal lobes on the right (14 × 24 mm, 7 × 16 mm), subperiosteal abscesses of both eye sockets (Fig. 2, a and 2, b).

Antibacterial therapy was adjusted: cefepime and amikacin, without positive dynamics. After 7 days, the patient was consulted by an otolaryngologist.

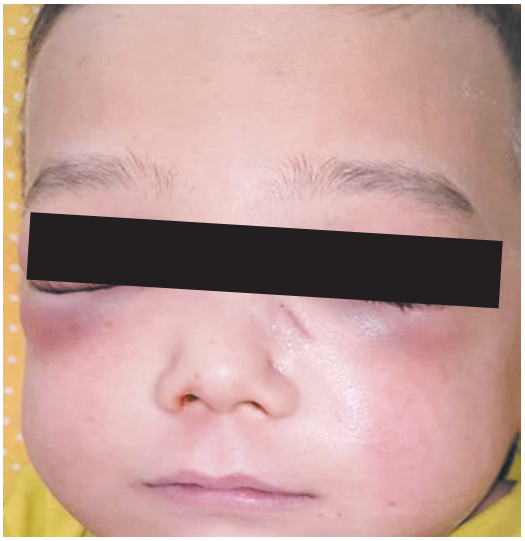

Fig. 1. Clinical manifestations: swelling, hyperemia of the skin of the lower eyelid of the left eye

Endoscopic examination of the nose: right nasal passage – the mucosa in the anterior parts was edematous, covered with black plaque in the form of dense crust, difficult to remove; left nasal passage – on the walls of the nasal cavity, there was a black formation, dense in structure, part of it was separated from the walls of the nasal cavity and removed, the bone tissue of the bottom of the nasal cavity on the left was exposed, necrosis of the inferior nasal concha on the left and middle nasal concha was revealed, the nasal septum was almost completely absent, the lumen of the nasal passages became passable up to the nasopharynx. On the oropharyngeal side, there was no mucosa on the hard palate 2.0 × 2.0 cm, the palate bone tissue was preserved.

Conclusion: necrosis of the nasal cavity with destruction of anatomical structures – the middle and inferior nasal concha on the left, the nasal septum, the lateral wall of the nasal cavity on the left, fungal infection? The formation was removed.

Antimycotic therapy was changed – voriconazole 400 mg/day was administered. Histological preparations of postoperative material revealed areas of necrotically altered tissue (mucosa) with fungal mycelium (mucormycosis?). The patient was consulted by the staff of the Department of Clinical Mycology, Allergology and Immunology at North-Western State Medical University. It was recommended to adjust the antimycotic therapy taking into account the histological examination results. Voriconazole was discontinued and liposomal amphotericin B 5 mg/kg/day was administered from August 23, 2021 (Fig. 3).

Based on clinical symptoms, CT scan of the brain and facial bones, and a histological report, a diagnosis was made: rhinocerebral mucormycosis with involvement of the paranasal sinuses, orbital tissues, facial bones, and brain. Acute pansinusitis. Abscess of the lower eyelid of the left eye (abscess drainage in August 6, 2021).

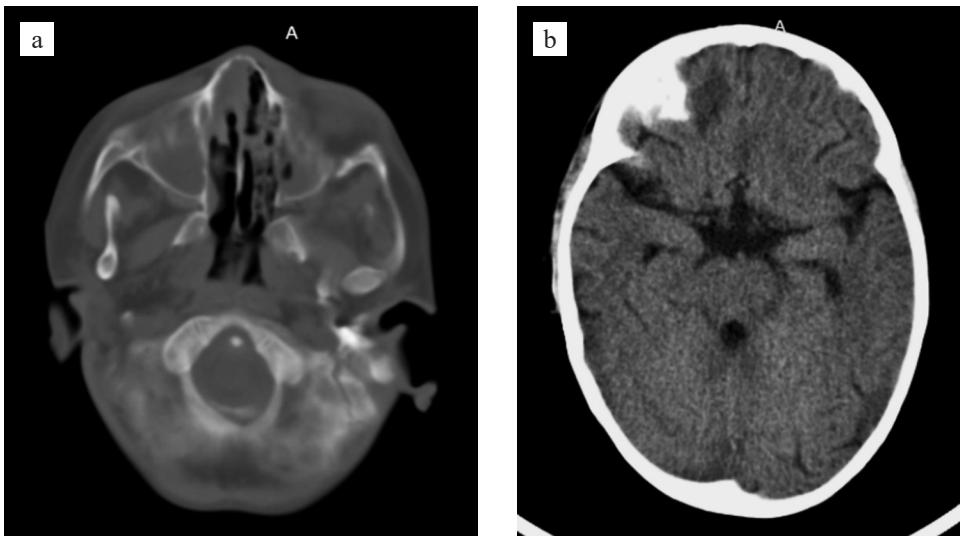

Fig. 2. CT scan: a, of paranasal sinuses; b, of brain

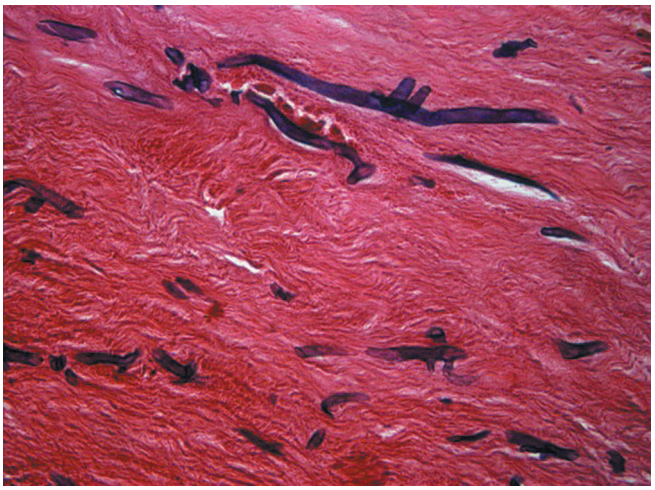

Fig. 3. Wide threads of non-septate mycelium in the postoperative material. Histological specimen: periodic acid-Schiff (PAS) staining; 400× magnification

Osteomyelitis of the bones of the nasal cavity, palate, alveolar processes of the maxillary bones, medial and lateral plates of the pterygoid process on the left side.

Antimycotic therapy was continued with liposomal amphotericin B at the same dose. The patient's general condition remained moderately severe with subfebrile body temperature. Two weeks later, brain CT and MRI showed negative dynamics in the form of increased volume of brain foci and progression of destruction of facial bones. Antifungal therapy was adjusted: the dose of liposomal amphotericin B 10 mg/kg/day was increased. Liver failure progressed during the therapy, and therefore the drug was replaced with isavuconazole 200 mg/day with a loading dose in the first 2 days. Over the next seven days, the patient's condition worsened: signs of systemic inflammatory response (sepsis) progressed, multiple organ failure increased and on October 7, 2021, the patient died on day 67 from the first clinical signs of invasive mucormycosis.

CLINICAL CASE #2

Patient O., 47 years old, in July 2022 came to the mycological clinic of Kashkin Research Institute of Medical Mycology, North-Western State Medical University with complaints of a significant decrease in visual acuity of the left eye, difficult nasal breathing, blood-stained purulent nasal discharge, periodic intense headaches.

From the patient's medical history, it is known that he was observed for a long time with chronic glomerulonephritis with an outcome of stage 5 chronic kidney disease (CKD) (d), corrected by chronic hemodialysis. The first kidney transplantation was performed in 2010. However, during the first year after transplantation, acute T-cell-mediated rejection occurred, which was controlled by pulse therapy with glucocorticoids (GCs). In 2017 (6 years after renal allotransplantation), humoral rejection occurred. By 2019, recurrent end-stage kidney disease in the graft was diagnosed. In April 2021, renal transplant nephrectomy was performed. In June 2021, repeat allotransplantation of a cadaveric kidney was performed. The patient received standard maintenance immunosuppressive therapy (tacrolimus, methylprednisolone, mycophenolic acid) to prevent graft rejection. In February 2022, he suffered a moderate COVID-19, complicated by viral pneumonia. He was on inpatient treatment. He received high GCs doses, antiviral drugs, broad-spectrum antibiotics, detoxification and oxygen therapy. Complete blood count revealed lymphocytopenia. The patient was discharged for outpatient treatment. After discharge from the hospital, he started complaining of intense headache, decreased visual acuity, and nasal breathing difficulties. He consulted an ophthalmologist and was recommended to go for a brain CT scan. CT scan revealed osteonecrosis of the upper jaw. The patient consulted an ENT doctor at his place of residence – necrotizing ethmoiditis and polysinusitis were diagnosed. In March 2022, endoscopic polysinusotomy was performed on the left side with decompression of the left orbit. According to results of cultures of the contents of the maxillary sinus on the left, bacteria were isolated and an antibacterial therapy course was administered. Histological examination detected osteonecrosis and signs of chronic inflammation. The patient's condition temporarily improved: the intensity and duration of headaches decreased, and nasal breathing was restored.

A month later, however, the patient's health began to deteriorate again: purulent nasal discharge with dark inclusions appeared, visual acuity in the left eye sharply decreased to complete blindness. MRI of April 2022: MRI picture of pathological infiltration with maximal changes in the retroorbital tissue, with involvement of the medial and superior rectus muscles of the eye, left optic nerve, probable destruction of the medial wall of the orbit and lesion of surrounding cells of the ethmoidal labyrinth extending to the superior orbital fissure area, left cavernous sinus and Meckel's space. Numerous focal changes in the white matter of the cerebral hemispheres, probably of vascular origin, foci in the paraventricular parts of the cerebellar vermis, persistent absence of contrast in the sigmoid sinus (consequences of thrombosis), signs of edema of the mucosa of the paranasal sinuses (ethmoidal labyrinth, lower frontal sinus and maxillary sinus on the left, condition after polysinusotomy on the left (Fig. 4).

He was hospitalized at Sechenov University in June 2022. Left maxillary resection with simultaneous bone grafting was performed. Revision of the left orbit and nasal cavity. According to microbiological examination of histological preparations, mycelium of fungi Mucorales spp. was detected. In connection with this, the patient visited the mycological clinic. The mycological clinic confirmed the diagnosis of mucormycosis with involve-

ment of the sinuses with bone destruction (B46.1) with involvement of retrobulbar tissue, destructive changes in the medial wall of the left eye socket and cells of the ethmoidal labyrinth on the left side. Considering the presence of kidney pathology, treatment with isavuconazole, repeated irrigation of paranasal sinuses, correction of immunosuppression (withdrawal of GCs), CT and MRI of the paranasal sinuses and brain once a month were recommended. Control cultures of nasal discharge and paranasal sinus aspirate were negative. After the treatment, control CT and MRI scans revealed remission of mucormycosis. The treatment for mucormycosis lasted for 12 weeks. The patient is under outpatient observation at the mycology clinic.

LITERATURE REVIEW

Infectious complications are the leading cause of death among patients within 1 year following organ transplantation (about 35%). In addition, they cause transplanted organ malfunction or rejection [4, 14, 15].

Micromycetes are opportunistic pathogens that can cause invasive diseases in critically ill patients as a result of a combination of several predisposing factors.

According to Livio Pagano et al., lung, heart and liver transplant recipients are considered to be at high risk of developing invasive fungal infections, while kidney transplant recipients are at lower risk of developing mycotic infection (Table) [16].

Published estimated incidences of mucormycosis in internal organ recipients have ranged from 0.4% to 16.0%, depending on the procedure and the geographical area [16]. Older studies found overall incidences of 0.2–2%, 0–2%, 0–3%, and 0–3% in kidney, liver, heart, and lung transplant recipients, respectively. A recent prospective multicenter TRANSNET study found that the 12-month cumulative incidence of mucormycosis was 0.07% in internal organ transplant recipients, with mucormycosis accounting for 2% of all invasive fungal infections [17]. Almyroudis et al. reported 10 cases of IOT-associated mucormycosis from their single institution and reviewed 106 other cases in the English-language literature from 1970 to 2002.

In this study, the transplanted organs were kidney (n = 73), heart (n = 16), lung (n = 4), heart and lung (n = 2), liver (n = 19), and kidney and pancreas (n = 2) [18]. At the same time, several researchers have shown that in

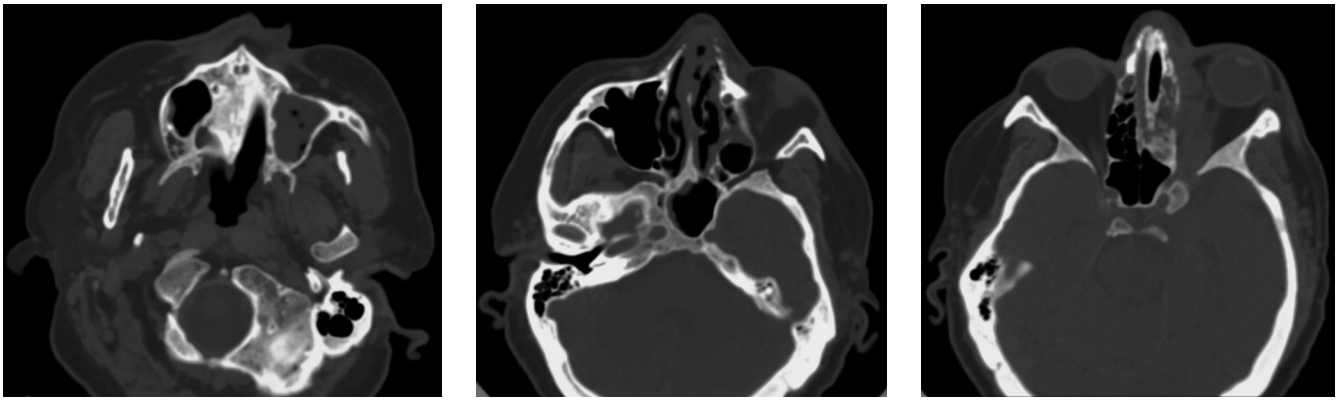

Fig. 4. MRI of the paranasal sinuses dated April 29, 2022. Inflammatory changes in the paranasal sinuses are most pronounced in the maxillary sinus on the left, with probable destruction of the medial wall of the orbit and lesion of the adjacent cells of the ethmoid labyrinth, spreading to the area of the superior orbital fissure, left cavernous sinus

Table

Categories of the degree of risk of developing invasive fungal diseases in accordance with morbidity and mortality rates

Low risk	Intermediate risk	High risk
Autologous HPSCT Hodgkin's lymphoma Chronic myeloproliferative diseases (CML and Ph conditions) Solid tumor Multiple myeloma Kidney transplantation Chronic immunologic diseases Systemic lupus erythematosus	Acute lymphoblastic leukemia Chronic lymphocytic leukemia Lymphoma COPD HIV/AIDS Myelodysplastic syndrome	Acute myeloid leukemia (primarily in the first induction) Allogeneic HPSCT (especially when cord blood is used) Heart, lung, liver transplantation

Note: HPSCT, hematopoietic stem cell transplant; CML, chronic myeloid leukemia; COPD, chronic obstructive pulmonary disease; HIV/AIDS, human immunodeficiency virus/acquired immunodeficiency syndrome.

some countries, there are higher rates of mucormycosis, ranging from 3% to 10%, which is associated with a larger number of immunocompromised patients [19]. Michael Osseis et al. analyzing the development of mucormycosis in liver transplant recipients, concluded that the frequency of infection in this patient cohort is 0.4%, with a mortality rate of 31–43% [20]. In a review by Rammaert et al., it was shown that 24% of patients with mucormycosis were organ transplant recipients [21]. This report demonstrates that mucormycosis can develop as a nosocomial infection associated with the transplant as a source of infection [21], as evidenced by the onset of clinical symptoms of the disease immediately after surgical intervention.

Mucormycosis is a late post-transplant complication [4]. The infection most often develops between 6 weeks and 12 months after transplantation (5–6 months on average) [15].

The main risk factors for mucormycosis in post-transplant patients are GvHD, use of GCs, renal failure, uncontrolled diabetes mellitus and previous use of voriconazole and/or echinocandins [15, 20, 21]. Interestingly, the use of tacrolimus has been associated with a reduced risk of mucormycosis in solid organ recipients, although they are usually potent immunosuppressants [22]. The role of calcineurin in the pathogenesis of invasive candidiasis and aspergillosis has been proven, but its exact role in the pathogenesis of mucormycosis has not been fully elucidated [22, 23, 24]. Calcineurin inhibitors and antifungals (amphotericin B) have been found to have synergistic or additive effects against *Mucorales spp.* [22]. Another important risk factor for mucormycosis is increased free iron in the bloodstream [25], which is most common in liver transplant recipients [20].

The most frequent clinical manifestations of mucormycosis are mycotic pneumonia [13–56%], rhino-orbital mucormycosis (26–87%), and skin lesions (22–13%) [14, 17, 18, 26, 27]. Focal CNS lesions develop in 2–5% of patients [14, 28]. Disseminated mucormycosis can develop in 26% of solid organ transplant recipients, most often in liver transplant recipients [20].

Clinical manifestations of mucormycosis are nonspecific and depend on the way mucormycetes penetrate the patient's body. For instance, inhalation of spores leads to mycotic sinusitis or mycotic pneumonia. When spores enter the gastrointestinal tract with food, necrotizing colitis and ileitis may develop [6]. The introduction of spores into soft tissues during trauma, maceration, and dressings leads to a localized skin process. The infection can spread to nearby tissues and organs if timely etiotropic treatment is not carried out. Dissemination through the bloodstream is possible [6, 7, 11].

The course of mucormycosis of the paranasal sinuses is similar to bacterial sinusitis or inflammation of the para-orbital tissue. Patients most often complain of headaches, paresthesias, pain over the area of the corresponding sinus, often irradiating along the course of the trigeminal or facial nerve; later, discharge from the nasal passages with streaks of blood appears. Swelling and hyperemia of mucous membranes, skin and soft tissues of the face increase [6, 14]. Patients are also bothered by pain in the eyeball and impaired skin sensitivity. There is a progressive decrease in visual acuity as a result of involvement of the ocular nerve in the infectious process or damage to the arterioles, which eventually leads to blindness and/or retinal infarction [10, 14, 19]. Examination reveals an ulcerative defect of the nasal or sinus mucosa with a focus of necrosis and an area of perifocal inflammation. The necrosis area increases daily, forming a "black scab" [6, 10]. At the same time, there may be no fever: only 50% of patients report an increased body temperature [14, 18]. The infection may then spread to the central nervous system. Nasal bleeding may be the first sign of infection penetration through the dura mater into the brain [6].

Clinical manifestations of pulmonary mucormycosis also require a differential diagnostic approach to clarify the genesis of the disease. Patients often present with fever (38–70%), persistent cough (50–61%), chest pain (22–37%), shortness of breath (19–34%) and hemoptysis (16–28%). There may be no increase in body temperature in neutropenic patients and organ transplant recipients receiving immunosuppressive therapy (10–15%) [6].

Mucormycosis of the skin and soft tissues is characterized by dense infiltrates that change the skin color from bright red to purple. Later, ulcerative defects with erythematous halo or subcutaneous nodules develop, merge and form necrotic areas ("black scab") [6, 8].

Mucormycosis of the gastrointestinal tract most often manifests itself as pain syndrome of varying intensity, abdominal bloating and other dyspeptic manifestations (nausea, vomiting), blood in stool may be detected. Laparotomy (for therapeutic or diagnostic purposes) reveals necrosis of intestinal tissues, intraperitoneal abscesses, and peritonitis [6, 9, 14].

Disseminated mucormycosis most often develops in liver transplant recipients and is manifested by prolonged elevations in body temperature above 38.50 °C, symptoms of secondary organ damage, where foci of dissemination and further development of signs of multiple organ failure are formed [6, 28].

Diagnosis of mucormycosis should be immediate, but this is hampered by the nonspecificity of clinical and radiographic signs.

First of all, it is necessary to rule out mucormycosis in recipients of internal organs with atypical sinusitis, pneumonia or fever of unknown origin. Diagnosis is based on the use of radiological and instrumental examination methods and detection of the pathogen in a material from the lesions.

The main radiological diagnostic method is high-resolution computed tomography. The most frequent

radiological signs of fungal damage to lung tissue are extensive lesions of lung tissue (involvement of several segments, lung lobes), subpleural foci, pleurisy, halo sign, crescent or reversed halo sign [6, 11, 14, 18]. Nonspecific signs include foci with indistinct contours, alveolar infiltration, and "frosted glass" changes. These symptoms are described in more than 50% of patients, they are not pathognomonic, as they are described in other mycotic lesions of lung tissue. When examining the paranasal sinuses by CT, the most commonly visualized area of the lesion is a zone of filling or tissue deficiency. As the process progresses, a bone destruction zone is determined. MRI is used if CNS lesions are suspected. In this case, single or multiple abscesses with a perifocal area of edema are more often detected [6, 11, 14, 18, 28, 29]. Serologic diagnostics for mucormycosis have not been developed.

The main methods of diagnostics of mucormycosis are mycological (microscopy, culture, histological) examination of material from the lesion [6, 14, 26, 30]. Microscopy of both native and stained preparations is performed. Most often, the smear is stained with calcofluor white. The stained preparation is microscoped using a microscope immersion system (900×, eyepiece 10×, lens 90×). This reveals a characteristic broad (10–50 μm) non-septate or sparsely septate mycelium branching at right angles. However, biopsy and culture of tissue samples from the lesion are frequently required because of the low diagnostic value of microscopy and culture of nasal aspirate, sputum, and bronchoalveolar lavage. Culture is performed in two repetitions, taking into account the different temperature regimes for growing filamentous fungi (37 °C and 28 °C), always at three points in the center of the dish. Incubation lasts for 10–14 days. Histological examination of the material reveals necrotizing abscesses and infarctions, inflammatory infiltration. Mucormycetes in tissues stain relatively well with hematoxylin and eosin, but additional staining, such as the PAS method or Gomori-Grocott silver impregnation, is often required [6, 11].

According to researchers, the pathogen is isolated in culture in 34–92% of patients. The most frequent causative agents of mucormycosis are: *Rhizopus spp.* (66–35%) and *Mucor spp.* (37%), *Lichtheimia spp.* (13%) [14, 31].

The mortality rate of patients with mucormycosis who did not receive systemic antimycotic therapy reaches 100%. Currently, the following groups of antimycotics are used to treat mucormycosis: polyenes (liposomal or lipid amphotericin B) and triazoles (posaconazole, isavuconazole). Administration of liposomal or lipid complex of amphotericin B at a dose of 5 mg/kg/day (AII) is recommended as a starter therapy, or 10 mg/kg/day for CNS lesions. The use of posaconazole and isavuconazole as starter therapy is less effective. Nevertheless, it is possible to use them in the form of infusion solutions if nephrotoxicity develops. Amphotericin B deoxycholate is not currently recommended for use [11]. The overall mortality in patients with mucormycosis treated with amphotericin B deoxycholate ranges from 39% to 57% [28]. An analysis of mucormycosis cases in organ transplant recipients confirmed the efficacy of therapy in 72% and 69% when lipid complex and liposomal amphotericin B were used as starting therapy [28]. The effect of antifungal therapy should be assessed on days 4–7. Additional CT or MRI scan is performed to visualize the focus of inflammation, and biochemical tests to assess the activity of the inflammatory syndrome. If initial treatment is ineffective, drugs from another group of antimycotics or combinations of drugs with different mechanisms of action are used, for example, liposomal amphotericin B and caspofungin, lipid amphotericin B and posaconazole [6, 11].

Antifungal therapy is continued until the clinical signs of the disease disappear, the pathogen is eliminated from the infection focus, radiologic signals start to return, and the immunosuppressive period is over. The average duration of treatment until the patient's condition stabilizes is 30–45 days, and it might take up to 180 days to reach complete remission. Antifungal medication is usually continued for at least 3 months. However, longer treatment or administration of secondary antimycotic prophylaxis is necessary in patients with persistent immunosuppression, such as with GvHD in organ recipients [6, 11].

Management strategies for patients with mucormycosis include correction of risk factors (recovery from ketoacidosis, withdrawal of immunosuppressive drugs, restoration of leukocyte levels in the peripheral blood, etc.) and use of surgical intervention – removal of affected tissues (necrectomy, lung lobe resection, pneumonectomy, maxillotomy, intestinal resection, etc.), in combination with antimycotic therapy with targeting drugs – level of evidence (AII) [6, 11].

CONCLUSION

Of all invasive fungal infections, mucormycosis accounts for 2–8% among solid organ recipients. Overall mortality rate reaches 38–48% in this patient cohort. Given the growing number of immunocompromised patients after solid organ transplantation and the existence of risk factors for the development of systemic fungal infections, transplant physicians should promptly conduct a diagnostic search algorithm to determine the best course of treatment for mucormycosis all in collaboration with a mycologist. Mycological vigilance by physicians, early biopsy and administration of targeted antimycotic therapy in conjunction with surgical procedures will optimize patients' prognosis and even save their lives.

The authors declare no conflict of interest.

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RAPID SUPPRESSION OF HBV REPLICATION BEFORE LIVING DONOR LIVER TRANSPLANTATION IN A PATIENT WITH HDV SUPERINFECTION. CLINICAL CASE REPORT

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Chronic hepatitis B virus (HBV) infection is one of the main problems of modern transplantology and transplant hepatology, often leading to potentially fatal complications. The only definitive treatment for HBV-related cirrhosis is liver transplantation. However, recurrence of HBV after transplantation may jeopardize both recipient and graft survival. Therefore, all HBsAg-positive recipients should receive prophylactic therapy with nucleos(t)ide analogues with or without hepatitis B immune globulin (HBIG), regardless of the hepatitis B e-antigen (HBeAg) status and HBV DNA level before transplantation. However, HBIG therapy has a number of disadvantages, and nucleos(t)ide analogues do not inhibit replication of super and co-infection. In addition, there is no unified understanding of the time limits for achieving a virologic response. In our clinical case, we report a rapid suppression (5 days) of high HBV (560,000 copies/mL) viral load in a patient suffering from HBV- and HDV-related cirrhosis, who was operated on with positive HBeAg at the time of transplantation. In our study, the use of standard therapy tenofovir disoproxil fumarate reduced the HBV viral load titer to undetectable values. In turn, given the positive HBeAg at the time of transplantation, HBV infection recurred in the early post-transplant period, which was eliminated without the use of HBIG therapy. The use of tenofovir disoproxil fumarate makes it possible to plan transplantation for patients with positive replication and high viral load, avoiding the use of HBIG, against the background of limited liver transplant wait time.

Keywords: liver transplantation, hepatitis B, hepatitis D, tenofovir disoproxil fumarate, tenofovir alafenamide, nucleos(t)ide analogues.

INTRODUCTION

Chronic hepatitis B virus (HBV) is one of the primary problems of modern transplantology and transplant hepatology, often leading to potentially fatal complications, including cirrhosis and hepatocellular carcinoma. Liver transplantation (LT) is the only definitive treatment for the above complications. However, recurrence of HBV infection after LT may compromise both recipient and graft survival, severely worsening treatment outcomes [1]. In addition, HBV is a necessary basis for hepatitis D virus (HDV) infection. HDV further aggravates the prognosis of chronic progressive liver disease, reducing overall survival and shortening the transplant-free survival period.

According to the latest international analysis by the Global Disease Burden, published in the Journal of Hepatology, 296 million (228–423 million) people are living with chronic HBV infection. And despite the availability of antiviral drugs, no country is yet on track to eliminate HBV infection by 2030, as outlined by the World Health Organization (WHO) and the European Association for the Study of the Liver (EASL). HDV prevalence, estimated at 12.0 million people (8.7–18.7), further clouds the statistics [2].

In turn, despite numerous studies and pharmacological developments aimed at reducing the incidence of HBV and NDV infection, including the presentation of bulevirtide, which was supposed to be a "rescue drug," HBV incidence remains high and bulevirtide is yet to receive FDA approval [3]. Thus, we currently have only two approved drug groups – nucleos(t)ide analogues (NAs) and pegylated interferon [4]. At the same time, both of these pharmacologic groups have drawbacks and limitations, and LT is still the only definitive treatment option for patients with advanced cirrhosis.

The presence of detectable HBV in the blood and/or positive HBeAg before LT are independent risk factors for recurrent HBV infection after LT. In addition, concomitant HDV infection and low patient compliance are additional risk factors for HBV relapse [1]. Therefore, pretransplant eradication of HBV infection is a universally recognized measure aimed at reducing the incidence of

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post-transplant complications. Moreover, according to recent clinical guidelines, all HBsAg-positive recipients should receive prophylactic NAs therapy with or without HBIG, regardless of pretransplant HBeAg status and HBV DNA level before transplantation [5].

However, as it has already been noted, HBIG therapy has a number of drawbacks. Besides, there is no unified understanding of the time limits for achieving virological response, and the results of achieving seroconversion and negative PCR differ significantly. According to a report from Marcellin P. et al., published in the New England Journal of Medicine, tenofovir disoproxil fumarate (TDF) resulted in HBV suppression rate of 76% and 93% in HBeAg-positive and HbeAg-negative patients at week 48, respectively [6]. In turn, the results of a phase III, double-blind RCT NCT01940471 comparing the efficacy of TDF and TAF published in The Lancet showed that HBV suppression at week 48 was achieved in 64% of HBeAg-positive patients. However, only patients with viral load >112,000 copies/mL were included in the analysis [7].

In our clinical case, we report a rapid suppression (5 days) of high HBV viral load in a patient with positive PCR titers of HBV (560,000 copies/mL), HDV (9,500 copies/mL) and positive HBeAg at the time of transplantation.

CLINICAL CASE REPORT

A 38-year-old female patient presented to the liver transplant unit for the first time in January 2023. Diagnosis at the time of admission: cirrhosis due to HBV negative, HDV positive infection. Child–Turcotte–Pugh Class A (6 points). MELD 7. No clinically significant portal hypertension. Large natural splenorenal shunt (≈2.5 cm). Splenomegaly. Biochemistry tests: total bilirubin 25 μmol/L (normal: 3.4–20 μmol/L); AST 45 IU/L (normal: 1–40 IU/L); ALT 57 IU/L (normal: 1–40 IU/L); albumin 33 (normal: 35–55 g/L); alkaline 135 (normal: 44–146 IU/L); GGT 48 (normal: 0–30 IU/L). Serology:

Table 1

Recipient pre-transplant characteristics

Recipient characteristics							
Age (years)	**Weight (kg)**	**Height (cm)**	**BMI**	**Blood group**	**GRWR**	**HBV (copies/mL)**	**HDV (copies/mL)**
38	56	160	21	0(I) Rh^+	1.1	560,000	89,000
Laboratory indicators							
Coagulation				**Biochemical parameters**			
Parameters	**Result**	**Reference range**	**Unit**	**Parameters**	**Result**	**Reference range**	**Unit**
PT	**47.2**	70–120	%	**Total protein**	**59**	65–85	g/l
INR	**1.37**	0.9–1.3	SI	**Albumin**	**28**	35–55	g/l
aPTT	**43.8**	26–31	sec	**Glucose**	4.8	3.2–6.1	mmol/L
				Urea	4	2.5–8.3	μmol/L
Fibrinogen	**1.5**	1.8–3.5	g/L	**Creatinine**	70	62–115	μmol/L
Lipid profile				**Total bilirubin**	**37.7**	3.4–20.5	μmol/L
Parameters	**Result**	**Reference range**	**Unit**	**Direct bilirubin**	**14.2**	1.7–17.1	μmol/L
Cholesterol	3.3	<5.2	mmol/L	**ALT**	**62**	<42	ME/L
Triglycerides	0.8	<2.28	mmol/L	**AST**	**81**	<37	U/l
LDL	1.05	<3.3	mmol/L	**ALP**	158	<270	U/L
HDL	1.55	1.03–1.55	mmol/L	**GGT**	29	6.1–42	U/L
Complete blood count				**K**	4.3	3.6–5.4	
Parameters	**Result**	**Reference range**	**Unit**	**Na**	137	135–150	
HB	14	11.7–15.5	g/dl	**Ca^+**	2.2	2.0–2.6	mmol/L
WBC	**2.78**	4.1–10	10^9/L	**Mg**	1.1	0.7–1.2	mmol/L
RBC	4.28	3.5–5.5	10^9/L	**CRP**	1.5	0–5	U/L
PLT	**150**	180–320	10^9/L	**LDH**	221	81–234	U/L
MCV	97	81–100		**α-Amylase**	59	25–125	U/L

Note: aPTT, activated partial thromboplastin time; INR, international normalized ratio; PT, prothrombin time; HDL, high-density lipoprotein; LDL, low-density lipoprotein; HB, hemoglobin; WBC, white blood cells; RBC, red blood cells; PLT, platelets; MCV, mean corpuscular volume; ALP, alkaline phosphatase; GGT, gamma-glutamyl transferase; LDH, lactate dehydrogenase; CRP, C-reactive protein; LT, liver tranplantation; BMI, body mass index; GRWR, graft-to-recipient weight ratio; HBV, chronic hepatitis B virus; HDV, hepatitis D virus; LDH, lactate dehydrogenase; ALT, alanine transaminase; AST, aspartate transaminase; ALP, alkaline phosphatase; GGT, gamma-glutamyl transferase; K, potassium; Na, sodium; Ca^+, calcium; Mg, magnesium.

positive HBsAg and anti-HDVAg. HBV PCR (quantitative, qualitative analysis) negative. HBeAg negative. HDV PCR: 12,000 copies/mL. The patient has been taking TDF 300 mg for the past two years, once a day. Considering the compensation of the process, the patient was recommended outpatient follow-up. After 6 months of follow-up, several episodes of hepatic decompensation were noted, manifested by a decrease in albumin to 28 g/L, an increase in serum total bilirubin to 37.7 μmol/L, and ascites formation (Table 1). Due to disease progression and transition to subcompensated stage (Child–Turcotte–Pugh class B (7)), MELD 3.0–15, she was recommended to undergo related liver transplantation.

The examination revealed a relapse of HBV infection: HBV 560,000 copies/mL; HBeAg positive. HDV PCR 9,500 copies/mL. Self-induced withdrawal from TDF was identified and confirmed. Given the known high antiviral efficacy of TDF and high barrier to HBV resistance [8], the administration of TDF 300 mg, once a day was resumed. A repeat blood PCR was obtained 5 days later, showing a more than 1000-fold reduction in viral load, less than 500 copies/mL. A subsequent PCR test performed 10 days after starting TDF, revealed no detectable HBV infection by quantitative and qualitative assays. However, the patient's HBeAg remained positive (Table 2). Given the latest published clinical protocols that allow for LT in HBeAg-positive patients, the patient underwent related LT.

***Transplantation:** The donor was the patient's 38-year-old sibling, with negative virological and serological HBV at the time of LT. In accordance with local protocol, as well as recommendations from recent publications, HBIG prophylaxis was not administered. Immunosuppression protocol: methylprednisolone 1000 mg intra-*

Table 2

Dynamic virological and serological indicators

Pre-transplant assessment (17 days before LT)				**During LT**			
Virology				**Virology**			
Parameters	**Result**	**Reference range**	**Unit**	**Parameters**	**Result**	**Reference range**	**Unit**
HBV (qty.)	**560,000**	Negative	copies/mL	HBV (qty.)	Negative	Negative	copies/mL
HBV (qual.)	**Positive**	Negative		HBV (qual.)	Negative	Negative	
HDV (qty.)	**9,500**	Negative	copies/mL	HDV (qty.)	Negative	Negative	copies/mL
HDV (qual.)	**Positive**	Negative		HDV (qual.)	Negative	Negative	
Serology				**Serology**			
Parameters	**Result**	**Reference range**	**Unit**	**Parameters**	**Result**	**Reference range**	**Unit**
HbsAg	**>100**	<1		**HbsAg**	**>100**	<1	
antiHBsAg		<10	IU/mL	antiHBsAg	<3	<10	IU/mL
HbeAg	**22.5**	<15		**HbeAg**	**21.3**	<15	
AntiHBeAg	38.6	<100		AntiHBeAg	29.6	<100	
AntiHBcoreAg		<100		**AntiHBcoreAg**	**>500**	<100	

Post-transplant day 5			
Parameters	**Result**	**Reference range**	**Unit**
HBV	**230**	Negative	copies/mL

Post-transplantation period (10 days after LT)				**Post-transplantation period (22 days after LT)**			
Virology				**Virology**			
Parameters	**Result**	**Reference range**	**Unit**	**Parameters**	**Result**	**Reference range**	**Unit**
HBV (qty.)	Negative	Negative	copies/mL	HBV (qty.)	Negative	Negative	copies/mL
HBV (qual.)	Negative	Negative		HBV (qual.)	Negative	Negative	
HDV (qty.)	Negative	Negative	copies/mL	HDV (qty.)	Negative	Negative	copies/mL
HDV (qual.)	Negative	Negative		HDV (qual.)	Negative	Negative	
Serology				**Serology**			
Parameters	**Result**	**Reference range**	**Unit**	**Parameters**	**Result**	**Reference range**	**Unit**
HbsAg	**>100**	<1		HbsAg	**>100**	<1	
antiHBsAg		<10	IU/mL	antiHBsAg	<3	<10	IU/mL
HbeAg		<15		HbeAg	3.1	<15	
AntiHBeAg		<100		AntiHBeAg	10.7	<100	
AntiHBcoreAg	**>500**	<100		**AntiHBcoreAg**	**>500**	<100	

Note: HBsAg, hepatitis B surface antigen; HBeAg, hepatitis B e-antigen; antiHBsAg, hepatitis B surface antigen antibody; AntiHBeAg, hepatitis B e-antigen antibody; AntiHBcoreAg, hepatitis B core antigen antibody; LT, liver transplantation; HBV, chronic hepatitis B virus; HDV, hepatitis D virus.

venously, intraoperatively, followed by conversion to oral form in the post-transplant period and maintaining a dosage of 20 mg a day; tacrolimus tablets at a dose of 1.0 mg once a day, starting from day 2 after surgery, with further increase up to 2.0 mg, once a day, keeping blood tacrolimus level within 8–11 ng/mL; mycophenolate mofetil 250 mg capsules, twice a day, on day 7 after surgery, with further increase up to 500 mg twice a day. Resumption of TDF orally was planned on day 8 after LT, but blood PCR on day 5 after surgery showed reactivation of HBV infection – 230 copies/mL. TDF 300 mg orally, once a day, was administered. On day 13 after surgery, control PCR (quantitative + qualitative) was negative for both HBV and HDV infection.

The post-transplant period was uneventful, the patient was safely discharged on day 19 after surgery.

DISCUSSION

HBV infection is a global public health problem. It is most prevalent in the Western Pacific and African regions [9]. Despite WHO's goal to eliminate viral hepatitis as a public health problem by 2030, annual global deaths from HBV are projected to increase by 39% from 2015 to 2030 if the status quo remains [10].

Nucleos(t)ide analogues (NAs) have significantly altered the clinical course of liver disease by halting the progression of liver injury and preventing HBV-related decompensated cirrhosis [11]. On the other hand, we still face some epidemiological challenges in global diagnosis of HBV infection, and effective measures aimed at preventing infection and disease progression are not always used rationally [10]. In addition, in patients with advanced cirrhosis, LT is still the only definitive treatment option. However, it is known that a high viral load before transplantation is associated with a high risk of HBV relapse after LT. In this regard, absence of HBV DNA is a necessary rule for all LT candidates [11].

At the same time, despite the prevalence of HBV infection and the existing risks of graft loss in case of HBV infection relapse, there is still no unified consensus among the global community on HBV relapse prophylaxis. For instance, combination therapy with high-dose HBIG and NAs has become standard in most European centers [12, 13]. However, the use of HBIG is subject to a number of serious limitations and drawbacks, such as high cost, risk of complications, and often limited availability. In contrast, the availability and high efficacy of third-generation NAs, such as ETV, TDF and TAF have led to the formation of alternative strategies for the prevention of HBV graft relapse aimed at avoiding the long-term use of HBIG therapy [13]. Today, an increasing number of hospitals, mainly located in Central Asia and Asia-Pacific region (India, China, Japan), are advocating the use of NAs monotherapy as an effective, safe, and simple tool to prevent post-LT recurrent HBV infection. At the same time, despite these reports advocating "mononucleos(t)ide" doctrines, the time limits for HBV eradication remain unclear, and are highly dependent on viral load, degree of diffuse liver injury, viral genotype, and many other factors.

Thus, in the present case, several eye-catching events are presented at once. In particular, a more than 1000-fold reduction in HBV infection (from 560,000 to 500 copies) was achieved in 5 days. HBV was completely eradicated in less than 10 days. In our opinion, additional data explaining such a rapid virological response can be provided by genotyping the described HBV. These studies, however, are available only in a few laboratories in the world.

In addition, even though a prolonged virological response was attained prior to transplant, recurrent HBV occurred as a result of the infection and the ensuing immunosuppression. However, TDF at a standard dosage was able to totally control this HBV, negating the need for HBIG.

CONCLUSION

The use of TDF allows planning for LT in patients with positive replication and a high viral load, without the need for HBIG, against the background of limited waiting time for LT. The case study highlights the remarkable efficacy of NAs monotherapy in preventing pre-transplant recurrent HBV as well as in treating the illness right away.

The authors declare no conflict of interest.

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POST-LIVER TRANSPLANT BILIARY COMPLICATIONS

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Biliary complications (BCs) are the most frequent complications following liver transplantation (LT). They are a major source of morbidity after LT. The incidence of BCs after LT is reported to range from 5% to 45%. The main post-LT biliary complications are strictures, biliary fistulas and bilomas, cholelithiasis, sphincter of Oddi dysfunction, hemobilia, and mucocele. Risk factors for biliary complications are diverse. In this article we seek to review the main types of biliary complications and modern approaches to their diagnosis and treatment.

Keywords: liver transplantation, biliary complications.

INTRODUCTION

Since the first experiences with liver transplantation (LT) in 1963 by Thomas Starzl, this procedure has become the standard treatment for end-stage liver disease [1]. Despite organ shortage, the number of orthotopic liver transplants (OLT) has continued to increase. According to the American Liver Foundation, 9,234 liver transplants were performed in 2021 in the United States alone, and over 35,000 worldwide [2–3]. In the Republic of Uzbekistan, more than 40 LT have been performed to date [4].

However, with the increasing number of transplants, this patient cohort continues to experience the problem of postoperative biliary complications (BCs). BCs are the most common complications following LT. They are a major source of post-LT morbidity and their frequency, according to various sources, ranges from 5% to 45% [4–6]. As surgical techniques continue to improve, the rate of BCs following LT has been decreasing, but they still remain a major source of morbidity and mortality in liver recipients. BCs are the Achilles' heel of LT, and represent pressing problems all over the world [5, 6].

BCs following LT include strictures, jaundice, cholelithiasis, and sphincter of Oddi dysfunction. Type of biliary reconstructions, bile duct ischemia, reperfusion injury, hepatic artery thrombosis, cytomegalovirus infection, and primary sclerosing cholangitis are some of the risk factors that influence the rate of BCs [7–8]. This review examines the main types of BCs, risk factors for these complications, and modern approaches to their diagnosis and treatment.

DIAGNOSIS OF BILIARY COMPLICATIONS AFTER LIVER TRANSPLANTATION

The presentation of BCs varies considerably. Some complications such as bile leaks may occur immediately in the post-operative period, while others may take weeks to develop. The clinical presentation can vary from asymptomatic patient with moderate liver enzyme elevations to a septic patient. Whenever a biliary complication is suspected, work-up usually begins with laboratory evaluation and an abdominal doppler ultrasound. Abdominal ultrasounds are relatively inexpensive, and are easy to perform (Fig. 1). Abdominal Doppler ultrasound of liver vessels allows differential diagnosis between biliary and vascular complications [9]. The positive predictive value of abdominal ultrasound is very high, especially in the presence of dilated bile ducts. In the absence of dilated bile ducts, the sensitivity of the ultrasound for detecting biliary obstruction ranges from 38% to 68% according to various sources [10].

Depending on which diagnostic technique makes the most sense to use, either magnetic resonance cholangiopancreatography (MRCP) or endoscopic retrograde cholangiopancreatography (ERCP) may be performed if ultrasound is unable to detect signs of bile duct dilatation despite a clinical suspicion.

It is preferable to perform ERCP in patients with biliobiliary anastomoses of bile ducts, as this allows for

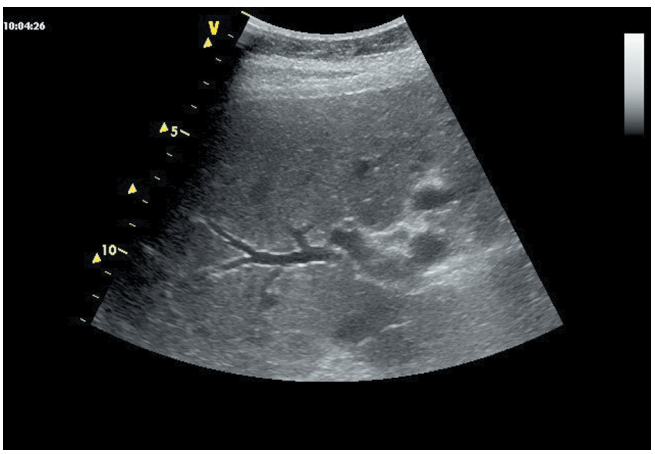

Fig. 1. Ultrasound imaging of intrahepatic bile duct dilation [5]

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therapeutic manipulation to be performed, such since papillosphincterotomy or stenting of bile ducts, removal of mechanical obstruction of bile ducts (lithoextraction), etc. Percutaneous transhepatic biliary drainage with cholangiography, or percutaneous transhepatic cholangiography (PTC), is used in rare cases where none of the aforementioned techniques may be used.

ERCP is technically very difficult to perform in biliodigestive anastomosis, so magnetic resonance (MR) cholangiography is preferable in such patients for diagnosis. However, in recent years, with the development of medical technologies, enteroscopes have become available and allow for endoscopic biliary examinations even in biliodigestive anastomosis [11, 12]. However, it may not be possible to use this strategy in all patients due to unfavorable surgical anatomy, adhesions, limited maneuverability of the endoscope, and the limited number of small caliber instruments that can be used through these endoscopes. In addition, these procedures require high skill and experience, and the learning curve is complex and therefore only available at specialized centers.

Another specialized method that has now been tested is gastrostomy by surgical or percutaneous means using endoscopic ultrasound followed by ERCP through the gastrostomy port [13].

MR cholangiography has excellent sensitivity (93% to 100%) in detecting biliary strictures. Based on MRCP data, it can also offer a road map for the endoscopist in planning the necessary intervention. Another advantage of MRCP is that this technique is noninvasive and does not create additional risks for the patient, unlike ERCP [14].

BILE LEAKAGE AFTER LIVER TRANSPLANTATION

Bile leaks (biliary fistula), along with bile duct strictures, are the most frequent post-OLT complications. Biliary fistulas, according to world reports [4–8, 15–18], occur in 2% to 35% of liver recipients. They can be classified in two categories [15–18]:

– Early (presenting within 4 weeks of OLT);
– Late (presenting from 5 weeks of OLT and beyond).

Etiology. Early bile leaks after LT usually occur due to anastomotic leakage, ischemia-reperfusion injury, infection, or after T-tube removal. Also, they can be caused either by coagulative necrosis (when bile ducts are damaged by an electrocoagulator or bipolar forceps) [14, 19].

Presentation. Bile leak should be suspected in any patient presenting with abdominal pain, fever, or having any peritonitis after LT, especially after T-tube removal. Bile leaks not related to T-tube removal typically present within the first 30 days after LT. Some patients, especially those on corticosteroids, may be asymptomatic, with no signs of pain or fever. In such cases, any unexplained elevations in serum bilirubin, fluctuation in liver transaminases, or the presence of free fluid accumulation in the abdominal cavity on ultrasound should raise suspicion for a bile leak [20].

Treatment of biliary leaks (biliary fistula). If a biliary fistula is suspected, it should be drained. Further tactics depend on the cause of the biliary leak. Thus, if bile leakage is associated with T-tube removal, ERCP and papillosphincterotomy are performed to increase bile flow resistance, a stent is placed in the defect zone. Additionally, complex antibacterial, analgesic, infusion and detoxification therapy is performed [15, 16]. Girotra et al. state that in the presence of end biliary anastomosis, most patients with biliary anastomosis can be treated endoscopically with papillosphincterotomy and bile duct stenting. The stent can remain in the bile duct for up to three months. After stent placement, the symptoms disappear quickly, but the actual healing of the leak may take up to 6–10 weeks [21].

Kochhar et al. report that in those cases where bile flow is associated with ductal ischemia – coagulative necrosis in the anastomosis area – the above-described technique cannot achieve such a good therapeutic effect. In such cases, the abdominal cavity is drained, a tube under the control of fistulography is installed in the defect area. Against the background of complex conservative therapy, such biliary fistulas close on their own. In rare cases, surgical intervention may become necessary to perform biliary reconstruction [20].

There is another method of treatment for BCs by nasobiliary drainage. For example, Thuluvath reports about successful closure of bile leaks using nasobiliary drainage [16]. Nevertheless, many authors consider that installation of an internal biliary stent provides better decompression from bile ducts into duodenum [15].

Biloma. Bile rupture and spilling of bile within the liver and abdominal cavity may result in the formation of a biloma (a cluster of bile surrounded by a pseudocapsule). Small bilomas, especially ones that communicate with the biliary tree, may resolve on their own. Bilomas are usually treated conservatively (antibiotic therapy). At the same time, biloma drainage options are available. In rare cases, open surgery to remove the biloma is required [15].

BILE DUCT STRICTURES

Biliary strictures (narrowings) are the second most common complication after LT. According to reports [4, 10, 13, 14, 18], the incidence ranges from 5% to 15% after deceased-donor LT, and 28–32% after living donor LT. Strictures are commonly seen as late complications, occurring approximately 5–8 months after transplantation, although there are cases of early postoperative strictures [10]. Post-LT biliary strictures are usually classified as anastomotic or non-anastomotic.

Anastomotic strictures (AS)

Strictures at the site of biliary anastomosis are the most frequent after OLT and can occur both in choledochojejunostomy and in choledochocholedochostomy [4, 18].

Causes of anastomotic strictures. The causes of AS are believed to include the following: inadequate mucosa-to-mucosa anastomosis, surgical technique, local tissue ischemia, and the fibrotic nature of the healing process [22]. Early bile leak after LT is also considered to be a risk factor for developing AS [15, 23]. In addition, vascular complications often lead to AS [9, 27]. In patients with T-tube, strictures at the choledochocholedochostomy anastomosis are often not typically evident until after removal of the T-tube [20]. A slight and transient narrowing of the biliary lumen occurs frequently in biliary anastomosis shortly after the LT due to postoperative edema. However, it is uncertain how many of these cases progress to clinically significant strictures [14]. In pediatric practice, the main risk factors for the development of strictures in liver fragment transplantation are impaired arterial blood flow, presence of an end-to-end biliobiliary anastomosis, and donor-side factors such as coagulation injury.

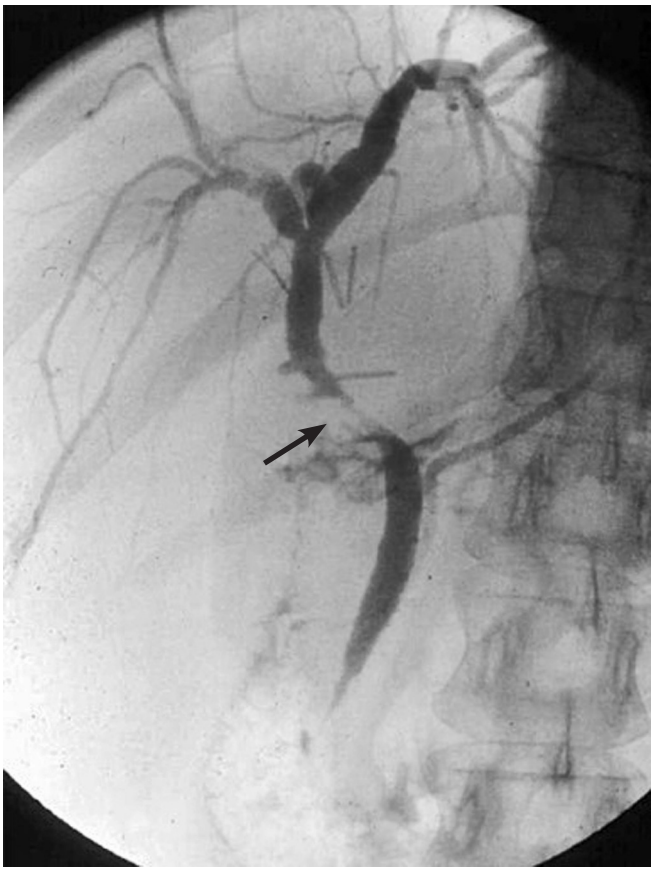

Fig. 2. Endoscopic retrograde cholangiopancreatography. Orange arrow indicates the anastomotic biliary stricture site [18]

Clinical presentation in anastomotic strictures. AS should be suspected in any liver transplant recipient who presents with jaundice, fever, abdominal pain, or even in patients with asymptomatic biochemical cholestasis. Bile duct dilatation can be observed with various imaging methods (ultrasound, MR cholangiography), however, it often does not develop immediately, so the absence of duct dilation is not a prerequisite for diagnosing strictures. When performing biopsy in such patients, histological findings may be suggestive of pericholangitis or bile duct proliferation [20].

Treatment of anastomotic strictures. Treatment varies depending on the type of biliary reconstruction performed on the patient during LT.

For example, in choledochojejunostomy biliobiliary anastomosis, it is advisable to perform ERCP followed by papillosphincterotomy and stenting of the common bile duct in the narrowing area (Fig. 2). Although outcomes vary markedly, studies have demonstrated good response to endoscopic therapy in over 75% of the patients [24, 25]. Endoscopic treatment is thus regarded as the treatment of choice for AS, especially in the choledochocholedochostomy group of patients. There are described techniques when the primary stent is replaced every three months with a larger diameter stent for a year, then the stent is removed permanently [20].

Sharma et al. report that when clinical AS occurs in the early postoperative period, the cause of jaundice and bile duct dilation may not be a true AS but postoperative edema, in such cases it is reasonable to perform ERCP and balloon dilation at the narrowing site; as a rule, this manipulation is enough to resolve obstructive jaundice [10].

It is also reported that SpyGlass technology has recently been actively used during ERCP for the treatment of strictures after LT. The Spyglass peroral cholangioscopy system is designed to be used by one operator rather than two operators as in the classical ERCP procedure. The system consists of two components: the SpyGlass fiber-optic probe (reusable) and the Spyscope access and delivery catheter, which is a single-use system. The instrument is inserted into the bile ducts through the duodenoscope's 4.2 mm diameter working channel. It has two main channels: a working channel for using forceps for biopsy or insertion of a 0.035-diameter guidewire, and a separate channel for the SpyGlass optical probe. The endoscopist operates the duodenoscope, optics, guidewire and probe simultaneously. This new technique not only allowed strictures to be clearly visualized and treated, but also simultaneously facilitated fairly easy and rapid cannulation, preventing the need for repeated ERCP/percutaneous access or surgery [28–30].

The rendezvous technique is employed when endoscopic bile duct cannulation is not feasible. This technique combines endoscopic technique with percutaneous transhepatic cholangiography (PTC) to facilitate bile

duct cannulation in cases where previous endoscopic attempts have failed. This integrated method increases the likelihood of success of biliary cannulation and facilitates the diagnosis and treatment of biliary diseases [65].

Intraductal magnetic compression is one of the newest techniques for treating biliary strictures endoscopically. Magnets are placed on the distal side of the stricture using an endoscope and on the proximal side using percutaneous access (Fig. 3). Then the magnets gradually move closer together and thus the stricture is resolved [31, 32]. Jang et al. reported that the overall clinical success rate of magnetic compression anastomosis for biliobiliary strictures was 87.5% of patients, and the recurrence rate was 7.1%. The clinical success rate of this method varies depending on the etiology of the stricture [33].

Ultrasound- and fluoroscopy-guided percutaneous transhepatic biliary drainage is performed in patients with biliodigestive anastomosis. Further, the drainage catheter (cholangiojejunostomy) during the year is replaced by similar ones with a larger diameter (bougienage).

In pediatric practice, percutaneous transhepatic bilioplasty is also used – it is a minimally invasive method, whose outcome is similar to that of surgical revision. For instance, several papers describing this technique have been published at the Shumakov National Medical Research Center of Transplantology and Artificial Organs [34–36]. A puncture needle was inserted into the dilated bile duct (primarily the second segment of the liver) under ultrasound guidance. A guidewire was used to try to pass the biliodigestive stricture, and if the stricture was successfully passed through the guidewire, an external-internal drainage catheter of 8.5 Fr diameter was installed, which, within over the next year, they were gradually replaced with drainage catheters of a larger diameter in order to bougienage the stricture (Fig. 4) [34]. In their results, the authors report that almost all patients in whom external-internal drainage catheter could be placed achieved a sustained response to this treatment modality after completion of the bougie course.

Although it has traditionally been considered that ERCP is virtually impossible to perform in patients

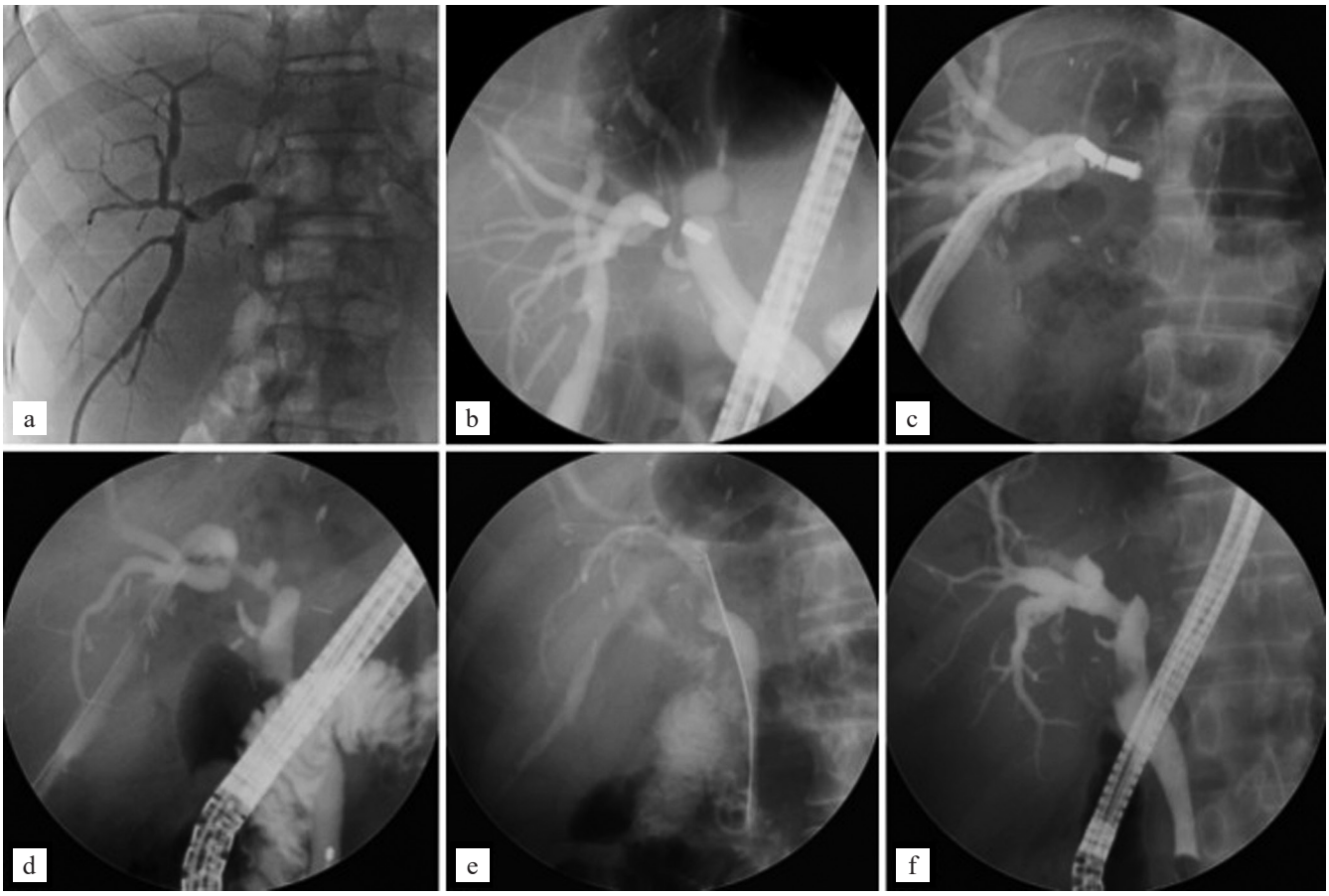

Fig. 3. Intraductal magnetic compression for stricture after liver transplantation from a living donor: a, dilated bile duct was cannulated by percutaneous transhepatic access; b, one magnet was placed via percutaneous access and the second magnet was placed by endoscopic retrograde cholangiopancreatography through the common bile duct. Magnet apposition was successful, and the percutaneous catheter was left in place to decompress the biliary tree; c, the magnets were removed by percutaneous transhepatic cholangioscopy; d, cholangiogram demonstrates recanalized biliary after removal of the magnets; e, a retrievable, fully covered, self-expandable metallic stent was placed for 6 months (replaced every 3 months); f, endoscopic picture after stent removal demonstrates a good effect from the procedure performed [33]

with biliodigestive anastomosis, many attempts have been made in the last decade to develop this surgical option in this patient cohort. For example, Arain et al. used elongated pediatric endoscopes to provide greater maneuverability during enteroscopy [37]. Japanese surgeon Tsujino describes balloon plasty and stenting of anastomotic biliary strictures in patients after LT using enteroscopy [38]. Also, a method is described when a percutaneous gastrostomy is formed using endoscopic ultrasound, and ERCP enteroscopy is performed through the formed access via a gastrostomy port. The authors explain the advantages of this technique by the possibility to work with standard duodenoscopes. In addition, as advantages, the authors note the convenience of maneuvering the endoscope during the procedure. The disadvantages of the procedure include additional surgical trauma [13].

AS, which are diagnosed earlier, are considered to be better amenable to therapy than strictures detected at a later date after LT. In cases where strictures cannot be treated with the above methods, surgical treatment – repeated biliary reconstruction – is performed [39].

Non-anastomotic strictures (NAS)

Non anastomotic strictures (NAS), also known as ischemic type strictures, are well known and have been described since the beginning of LT. They are frequently hilar in location, but can also be diffusely intrahepatic. Unlike AS, NAS symptoms tend to be longer and multiple on presentation. NAS incidence ranges from 5–15% with mean time to presentation of 3.3–5.9 months post-LT [40, 41].

Etiology. A few theories have been proposed for the development of NAS. Blood supply to the supraduodenal bile duct is predominantly from vessels which are resected during LT. The remaining blood supply to the donor bile duct then comes from the hepatic artery and its branches, which are tenuous and highly susceptible to

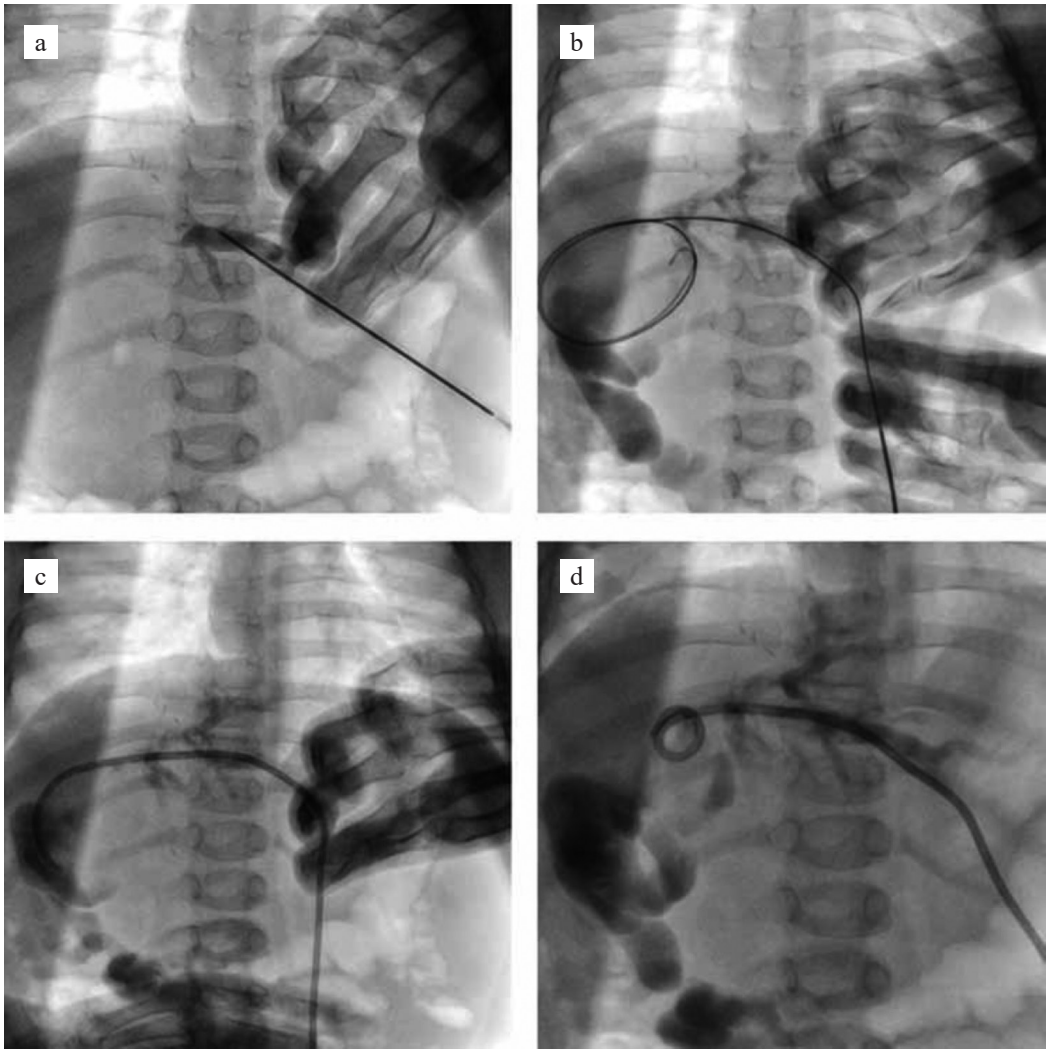

Fig. 4. Stages of placement of external-internal drainage catheter: a, contrast is injected through the Chiba needle into the dilated duct; b, guidewire is passed through the stricture into the efferent loop of the intestine; c, Dawson–Mueller drainage catheter through a guidewire is passed through the stricture; d, distal loop of the drainage catheter is placed in the intestine behind the stricture [36]

ischemic injury. In patients with NAS, up to 50% have demonstrable hepatic artery thrombosis. Prolonged cold ischemia time has also been shown to be responsible for the development of NAS. Besides ischemia, an immunological cause has also been proposed. This is mainly due to the observation of an increased incidence of NAS in cases with ABO-incompatible grafts, in patients with autoimmune hepatitis or primary sclerosing cholangitis. Thus, the causes of NAS are multifactorial and debatable [10, 42, 43].

Management. The presentation of NAS is similar to that of AS. NAS are more difficult to manage than AS, and treatment is often more "aggressive". In cases with early, revascularization should be attempted (in the presence of ischemia or hepatic artery occlusion), because prolonged ductal ischemia may lead to graft abscessation, resulting in the need for retransplantation [44].

In balloon dilation/stenting of strictures, treatment success depends largely on the number and location of strictures. Extrahepatic strictures generally respond better to therapy. If radiological and endoscopic therapies fail, open biliary reconstruction may become necessary. Success rates are higher if surgery is done within two years of LT and if the liver biopsy does not show any significant fibrosis [16, 38].

Retransplantation may also be considered in patients with treatment failure, or in the presence of secondary biliary cirrhosis, recurrent cholangitis, or progressive cholestasis [16, 38, 39, 44–46].

SPHINCTER OF ODDI DYSFUNCTION (SOD)

One of the common phenomena after LT is a mild increase in the size of donor and recipient common bile ducts. In certain cases, significant dilatation of both recipient and donor bile duct in association with biochemical abnormalities occurs in the absence of cholangiographic evidence of obstruction. In these cases, SOD is suspected. The incidence of SOD is reported to be up to 7% [17, 39, 47].

Etiology. The pathogenesis of SOD is attributed to denervation of the sphincter during OLT. This leads to an increase in basal pressure, thus causing increased pressures in the choledochal duct. Two types of SOD have been proposed: stenosis and dyskinesia. Any process that leads to chronic inflammation can lead to sphincter stenosis. Dyskinesia, on the other hand, is usually seen as a result of functional disturbance of the sphincter [22, 48, 49].

Management. There have been virtually no clinical trials that demonstrate the best treatment option for SOD. In recent years, endoscopic therapy (ERCP) with papillosphincterotomy with or without stenting has been the most acceptable treatment option for SOD [20, 21, 38, 47, 50].

BILIARY STONES, SLUDGE, AND CASTS AFTER LIVER TRANSPLANTATION

Bile duct obstruction by stones or sludge can occur at any stage following the OLT. Sludge is described as a thick collection of mucous, calcium bicarbonate and cholesterol crystals, which, when left untreated, can go on to form biliary stones. Sludge and casts usually occur within the first year of transplant, while stones tend to occur later on. Also, the so-called biliary cast is a complete encrustation of the bile duct by sludge or stones with the formation of a biliary cast [20, 21, 51]. Biliary cast disease is described in 3.3–12.3% of cases in patients after liver transplantation [21, 25].

Etiology. Theoretically, anything that increases the viscosity of bile or reduces flow can predispose to the formation of sludge, and stones. Bile duct mucosal damage due to obstruction, ischemia, or infection is thought to play a role in the development of cholelithiasis [21]. Of patients presenting with biliary stones and sludge after LT, most will have an underlying stricture. Among other things, immunosuppressants such as cyclosporine may play a role in bile lithogenicity by inhibiting bile secretion and promoting biliary stasis [20, 21].

Presentation. Patients commonly present with abdominal pain, cholestatic liver tests, and, possibly obstructive jaundice.

Management. According to reports, cholangiography is the only reliable imaging method for sludge, while ultrasonography and computed tomography (CT) scans are of limited value. If sludge alone is present, then it would be reasonable to first attempt medical treatment with ursodeoxycholic acid. ERCP with sphincterotomy, lithotripsy and stone extraction are successful in the presence of end-to-end choledochoduodenostomy.

According to Girotra et al., ERCP with sphincterotomy has high rates of success in removing gallstones and sludge in 90–100% of cases [21]. However, removal of biliary casts can be challenging and may require several procedures including sphincterotomy, extraction of casts with a balloon and/or basket, stent placement and lithotripsy, or may ultimately require PTC [61, 81]. In various studies, endoscopy has shown successful removal of biliary casts in 25–60% of patients [41, 52]. In fact, in cases of severe biliary necrosis and presence of casts, repeated endobiliary interventions using baskets and balloon dilatation are often necessary, and stent placement is usually not recommended in early stages because of the risk of occlusion by small residual stones and sludge [53].

In contrast, bile duct stones are usually easily removed with ERCP. Sometimes, proximal stones may present a problem, and in such cases, direct cholangioscopy can be performed to remove stones. In addition, if the filling defect is located proximal to an existing anastomotic stricture, treatment of the stricture becomes the first step

toward clearing the duct. Lithotripsy can be combined with stone removal procedure [21]. Direct cholangioscopy can be performed using ultrathin pediatric endoscopes that can be inserted directly into the bile ducts to study the ductal anatomy and remove stones and casts from bile ducts [37]. In addition, enteroscopy can be used to perform ERCP in patients with biliodigestive anastomosis to remove gallstones or casts [38].

However, in many patients with choledocholithiasis, the clinical course may be completely asymptomatic, which is often due to the fact that the graft is denervated. Patients often do not experience pain and fevers due to steroids and immunosuppressants after LT. Sometimes cholelithiasis can form in the background of strictures due to bile stasis and this occurs proximal to the stricture. In such cases, endoscopic treatment becomes difficult [21].

MUCOCELE

In rare cases, the donor's cystic duct can be included in the suture line of biliary anastomosis. As a result, a blind sac lined with mucous membrane is formed. Due to mucin accumulation, this sac may increase in size and cause bile flow obstruction. Endoscopy is often ineffective in these cases. Percutaneous drainage or surgical treatment are effective treatment options. Differential diagnosis of mucocele includes any type of fluid accumulation, such as bilomas, abscesses, hemorrhages, and aneurysms [46, 54].

HEMOBILIA

Hemobilia is a rare complication following liver transplantation. As a rule, it develops after biopsy of the graft or after percutaneous hepatic manipulations on the graft. However, cases of spontaneous hemobilia and hemobilia against the background of bile duct pseudoaneurysm rupture have been reported [9, 46, 55]. Treatment of hemobilia requires both hemostasis and treatment of obstructive jaundice, which is caused by blood clots. In some cases, bleeding spontaneously stops with supportive therapy and correction of coagulopathy. Embolization of bleeding vessels using interventional techniques is necessary if bleeding is permanently prolonged or there is a large blood loss. Removal of clots from the biliary tree to resolve the obstruction is usually performed endoscopically [56].

Shinjo et al. described an effective technique for resolving hemobilia by performing ERCP with thrombus extraction and nasobiliary placement. They consider this therapy to be the first choice. Nasobiliary drainage provides the possibility of biliary tract lavage, which prevents cholangitis and indicates the presence of recurrent bleeding. In most cases, a combination of endoscopic treatment (stenting) and hemostatic therapy gives good outcomes [57, 58].

KINKING OF THE COMMON BILE DUCT

Excessive length of the donor's common bile duct can lead to its kinking. Bile flow may be impaired as a result of this. The reported incidence is 1.6% among all OLTs. ERCP with placement of a long plastic stent usually resolves cholestasis. Biliary reconstruction is required in rare cases [59].

FOREIGN BODIES

Suture material or remnants of perforated tube can be sources of obstructive jaundice or stone formation. ERCP and PTC are effective methods for diagnosing and treating bile duct foreign bodies. Biliary reconstruction is required in very rare cases [46, 60].

RISK FACTORS FOR BILIARY COMPLICATIONS AND METHODS OF THEIR PREVENTION

Risk factors. There are many risk factors of BCs. The type of biliary reconstructions, bile duct ischemia, reperfusion injury, hepatic artery thrombosis, cytomegalovirus infection, surgical technique, variant biliary anatomy, biliary stasis, and primary sclerosing cholangitis (PSC) are some of the risk factors that influence the rate of these complications (Fig. 5) [7, 8, 26, 63]. Moreover, a recent systematic review of 45 articles (14,411 patients) showed that BCs develop more frequently in patients with MELD >25, PSC or malignancy [63, 100].

Living-donor liver transplant can be a risk factor for BCs. According to reports, various transplant centers compared the outcomes of living-donor and deceased-donor transplantations. In almost all studies, BCs developed more often in patients who received liver from living donors. The same applies to a comparison of the outcomes of related liver transplantation and split liver transplantation in both adults and children (see Table 1).

Type of biliary anastomosis is one of the main factors determining the risk of BCs after orthotopic LT. The two most common forms of biliary tract reconstruction are choledochocholedochostomy (anastomosis of the common bile duct of the transplant to the common bile duct of the recipient) and choledochojejunostomy (anastomosis of the bile duct to a part of the jejunum; such anastomosis is most often performed with a part of the jejunum taken out according to the Roux technique) [61]. The choice of the type of biliary reconstruction may be influenced by many factors, including an underlying disease of the recipient, diameter of the bile ducts of the donor and recipient, number of biliary ducts on the graft (for living donor transplantation), history of bile duct surgery, retransplantation, other intraoperative circumstances, as well as the preferences of the operating surgeon. There are no clear guidelines regarding the optimal type of biliary reconstruction, and many surgeons have their own opinion on this matter [20].

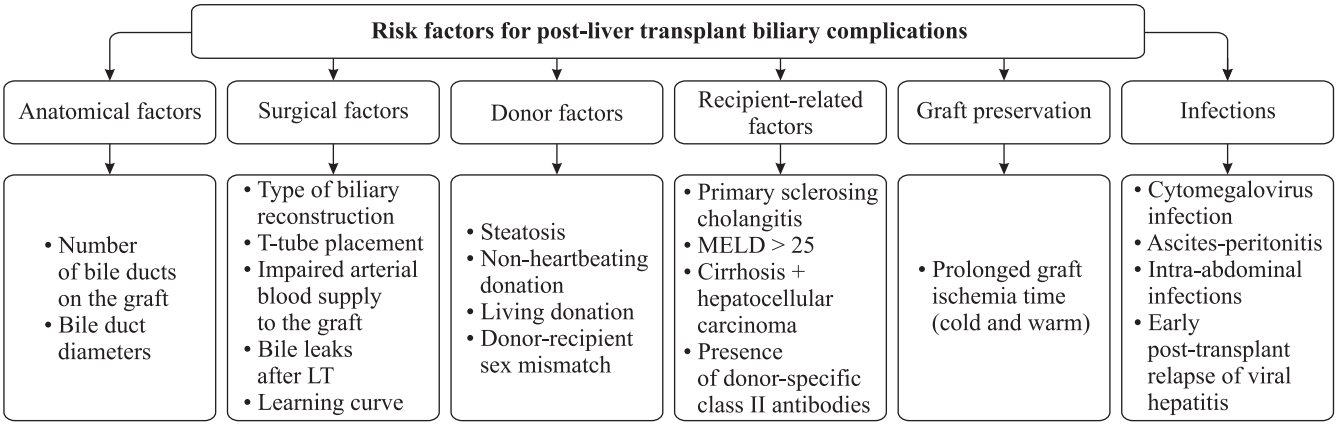

Fig. 5. Risk factors for biliary complications following liver transplantation

Table 1

Comparison of the number of biliary complications after split liver transplantation from living and from deceased donors

Study	Transplant type	Patient count	Age	MELD PELD	Biliary complications	
					Stricture	Bile leaks
Barbas et al. [84]	Living RL donor	48	54.7 ± 9.4	17.8 ± 8.7	3 (6.3%)	4 (8.3%)
	Deceased liver	128	56.7 ± 9.3	21.8 ± 10.3	4 (3.1%)	2 (1.6%)
Reichman et al. [85]	Living RL donor	145	54.2 ± 7.5	14.4 ± 3.8	26 (17.9%)	15 (13.3%)
	Deceased liver	145	53.9 ± 7.7	14 ± 6.8	16 (11%)	5 (3.4%)
Lei et al. [86]	Living RL donor	31	44.4 ± 9.7	9.3 ± 6.1	1 (3.2%)	1 (3.2%)
	Deceased liver	52	44 ± 8.2	9.1 ± 5.8	0	1 (1.9%)
Li et al. [87]	Living RL donor	128	43 ± 8.6	19.5 ± 10.7	12 (9.4%)	3 (2.3%)
	Deceased liver	221	44.5 ± 9.7	18.2 ± 9.6	3 (1.4%)	6 (2.7%)
Chok et al. [88]	Living RL donor	54	51 ± 12	40 ± 1.3	2 (3.7%)	0
	Deceased liver	40	51 ± 10.8	39 ± 1.3	1 (2.5%)	0
Liu et al. [89]	Living RL donor	124	47.5 ± 8.3	21 ± 6.5	31 (25%)	5 (4%)
	Deceased liver	56	48 ± 9.8	19 ± 10.8	3 (5.4%)	2 (3.6%)
Wan et al. [90]	Living RL donor	40	48.6 ± 9.7	–	7 (17.5%)	1 (2.5%)
	Deceased liver	80	49.5 ± 8.9	–	5 (6.25%)	1 (1.3%)
Hu et al. [91]	Living RL donor	389	48.1 ± 8.7	–	BL + AS – 81 (20.8%)	
	Deceased liver	6471	50.1 ± 9.4	–	BL + AS – 721 (11.1%)	
Kim et al. [92]	Living RL donor	21	53.1 ± 10.3	13.1 ± 5.4	2 (9.5%)	2 (9.5%)
	Deceased liver	29	51.3 ± 9.2	24.9 ± 11.6	0	0
E. Kim et al. [93]	Living RL donor	109	52 ± 8.5	12.5 ± 8.3	BL + AS – 10 (9.1%)	
	Deceased liver	76	53.2 ± 11	24.9 ± 11.7	BL + AS – 5 (6.5%)	
Jiang et al. [94]	Living RL donor	70	40.3 ± 8.2	23.9 ± 11.1	5 (7.1%)	4 (5.7%)
	Deceased liver	191	44.1 ± 9.3	21.7 ± 9.9	7 (3.6%)	8 (4.2%)
Latypov [95]	Living RL donor	22	17.9 ± 12.5	11.9 ± 8.5	0	5 (23%)
	Split DRL	22	21.9 ± 17.5	15.6 ± 10.2	2 (9.2%)	2 (9.2%)
	Living LLQ donor	22	1	1	0	4 (18.4%)
	Split DRL	22	1	1	0	4 (18.4%)
Dalzell et al. [96]	Living LLQ donor	508	1	1	1 (0.2%)	0
	Split DRL	403	1	1	2 (0.5%)	0
Yoon [97]	Living LLQ donor	56	1.4 (0.9–2.8)	–	BL + AS – 3 (4.3%)	
	Split LLQ	63	1.0 (0.8–7.8)	–	BL + AS – 721 16 (28.6%)	
Diamond et al. [98]	Deceased liver	1183	6.6	11.6	38 (3.2%)	44 (3.7%)
	Split LLQ	261	3.2	16.7	6 (2.2%)	41 (15.7%)
	Reduced liver	388	3.2	18.2	20 (5.1%)	46 (12%)
	Living LLQ donor	360	2.6	16.6	15 (5.2%)	53 (14.7%)

Note: MELD, model for end-stage liver disease; BL, bile leak; AS, anastomotic stricture; RL, right lobe; DRL, dilated right lobe; LLQ, left lateral quadrant.

In orthotopic cadaveric LT, biliobiliary anastomosis is the most common modality of biliary reconstruction. This technique is preferred because it is technically easier to perform and the sphincter of Oddi function is preserved in this type of reconstruction. Also, amidst bile duct complications after this type of reconstruction, it is possible to resolve the complication endoscopically with retrograde cholangiography (ERCP). In addition, preservation of the sphincter of Oddi theoretically reduces the risk of ascending cholangitis because it serves as a barrier against reflux of intestinal contents into the biliary tree [11].

Roux-en-Y hepaticojejunostomy is another type of biliary reconstruction. Usually, this type of biliary reconstruction is used in patients with a history of biliary diseases, such as primary sclerosing cholangitis, with previous surgical biliary interventions, in size discrepancy between the donor and recipient ducts. This type of anastomosis is also often used in related LT, in split LT or in pediatric LT [75, 76]. Compared to choledocho-choledochostomy, biliodigestive anastomosis requires more time to perform and does not provide adequate opportunity for endoscopic evaluation of the biliary system after LT [20]. Potential complications of biliodigestive reconstructions include intestinal perforation, bile duct stricture, anastomotic leak and bleeding at the site of interintestinal anastomosis [10].

Also, the technique of both biliobiliary and biliodigestive anastomosis may influence the incidence of complications. Many studies on living-donor LT have focused on the question of which suture to use: continuous or interrupted? Even if the outcomes are partially contradictory, it can be summarized that the incidence of biliary leakage increases with single sutures, whereas the incidence of stenosis increases with continuous sutures [68–69]. However, there are no randomized studies on this issue. In a retrospective study of 100 patients after LT, Castaldo et al. showed the outcomes of BCs with choledocho-choledochostomy. With an almost identical incidence of about 8% leak, anastomosis strictures were almost twice as common with continuous suturing (9.8% *vs.* 5.1%), but this was not statistically significant [70]. Kasahara et al. reported an increased incidence of strictures in choledocho-choledochostomy by using continuous suture, while the incidence of strictures in choledochojejunostomy was decreased [71]. However, the opposite outcome was observed by another working group [72] (Table 2).

The frequency of BCs is also influenced by donor biliary anatomy. Voskanyan et al. presented their own classification of biliobiliary and biliodigestive reconstructions used in transplantation of the right liver lobe from a living donor. Based on this classification, the authors calculated the risks of BCs when performing a certain type of anastomosis. A statistically significant risk of biliary bleeding was noted in ductoplasty (merging of two or more bile ducts into one mouth) and applying biliobiliary anastomosis after such plasty. The authors also noted that the patients with a history of bile leakage were at a higher risk of developing AS [27].

A similar outcome is demonstrated by Baker et al. [62]. A multicenter study was conducted, where the results and the number of BCs following related LT were analyzed. The researchers note that the rate of BCs is influenced especially by donor biliary anatomy. Thus, according to data from this study, in patients with complex variable anatomy of bile ducts, the rate of BCs can reach 76%, despite various surgical techniques (Fig. 6).

Routine placement of T-tubes in the formation of biliobiliary anastomosis is a risk of cholangitis and bile leakage after their removal. In a retrospective study by Olivier Scatton, which included analysis of 180 patients, it was demonstrated that the incidence of biliary fistula and risk of cholangitis was 10% in the T-tube group and

Table 2

Comparison of the incidence of bile leaks and anastomotic biliary stricture depending on the suturing technique in living related liver transplantation

Author	Total cases (n)	Suture	Cases (n)	Biliary effusion	Stricture
Kasahara et al. [71]	321	Interrupted	25	8%	36%
		Twisted	148	4.7%	25%
Soejuma et al. [72]	182	Interrupted	63		10% (BB); 31.8% (BD)
		Twisted	37		45.9% (BB); 0 (BD)
Hwang et al. [68]	282	Interrupted	259	5%	16.8% (BB); 15.8% (BD)
		Twisted	23	13%	21.7% (BB)
Tashiro et al. [72]	80	Interrupted	30	15%	6.7% 20%
Mita et al. [73]	231	Interrupted	50 48		9.5%
Marubashi et al. [69]	83	Interrupted	118 44	1.2%	7.2%

Note: BB, biliobiliary anastomosis; BD, biliodigestive anastomosis.

2.2% in the group without a T-tube. Also, the cumulative 3-year patient survival rate was higher in the group without a T-tube (80.1% *vs.* 72.8%), which the authors attributed to the higher complication rate among T-tube patients [82].

At the same time, German surgeon S. Weis and co-authors conducted a prospective randomized study of BCs following LT after bile duct anastomosis with or without T tubes. They found out that According to the results of the study, there was a significant increase in the complication rate in the group without a T-tube. So, the authors concluded that the usage of T-tubes is safe and an excellent tool for the quality control of biliary anastomosis [83].

According to a meta-analysis of 1027 patients, those without a T-tube had a lower incidence of cholangitis and peritonitis with an overall lower biliary complication rate. Interestingly, this meta-analysis found no significant differences between the groups of patients with and without T-tube insertion in terms of other complications such as biliary leak, hepatic artery thrombosis, and retransplantations. Mortality due to BCs did not differ in the same way [83]. On the contrary, a larger meta-analysis from 2021, which included a population of 2399 patients, showed that the use of T-tubes increased the incidence of strictures, biliary leak, and cholangitis in cadaveric liver recipients. The authors conclude that studies published in the last decade have not provided sufficient evidence to support the routine use of T-tubes in adult recipients [83].

Surgical risk factors for BCs also include vascular complications after LT [9]. The main contributor to the development of BCs is complications related to the liver transplant artery due to bile duct ischemia (Fig. 7).

Some authors have noted that a patient's baseline MELD is a risk factor for biliary strictures [23, 26, 64]. However, there are studies that refute this statement [65].

According to Egawa et al. [66], BCs are more common in female recipients. These data are inconsistent with the work by Voskanyan et al. where the researchers did not obtain statistically significant differences in the number of BCs in men and women, so the gender difference cannot be considered an unambiguous risk factor [26].

Donor-recipient gender mismatch has been reported as a risk factor for various complications, including biliary. A higher complication rate is noted in a pair where the donor is female and the recipient is male [26, 67].

New data suggest that immunosuppression regimens may influence the development of various BCs. For example, sirolimus administration has a higher risk of developing bile duct strictures, and cyclosporine may influence enhanced gallstone formation [21, 51].

Early recurrence of viral hepatitis after transplantation also increases inflammation and, consequently, the risk of strictures [21].

Methods of preventing biliary complications. Many transplant centers use perforated tubes of bile ducts when performing hepaticojejunostomy. It allows to estimate the quality of bile secreted by the transplant, and also such drainage is used to control strictures and bile leakage in the postoperative period [66, 77, 78].

Ando et al. performed frame drain of bile ducts at biliodigestive anastomoses in pediatric cohort recipients and reported only one stricture and one biliary leak out of 49 patients [78].

Monakhov A.R. and co-authors describe their experience of using frame drains in pediatric practice. They describe the experience of left lateral sector transplantation in 149 patients, where frame drainage was installed in 82 patients, and frame drainage was not used in 67 patients. It has been reported that BCs in the group without frame drain was 20%, which was higher than that in the group with external frame drain 8.5% [79].

However, the data on the use of frame drains are contradictory. For example, Japanese surgeon Egawa, analyzing 400 patients after LT, reports increased number of bile leaks when using frame drainage during biliodigestive anastomosis (16.6% *vs.* 10%), while the number of strictures decreased (8.2% *vs.* 9.6%) [66].

To reduce the frequency of biliary leaks in the pediatric cohort of patients, Gautier et al. suggest using peritonization of the left lateral sector of the upper lip of the biliodigestive anastomosis with the round ligament [80, 81]. A lower bile leak rate in patients who underwent

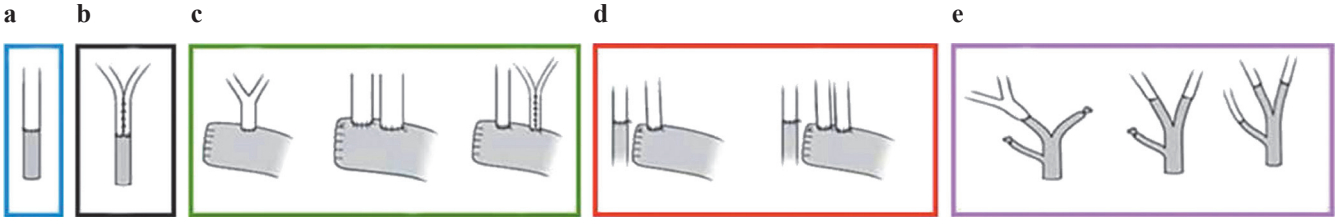

Fig. 6. Surgical techniques for biliary reconstruction for anatomic variant of bile ducts: a, choledocho-choledochostomy; b, choledocho-choledochostomy with ductoplasty; c, variants of biliodigestive anastomosis, including ductoplasty; d, a combination of choledocho-choledochostomy and biliodigestive anastomosis; e, variants of choledocho-choledochostomy for complex biliary anatomy of donor liver [62]

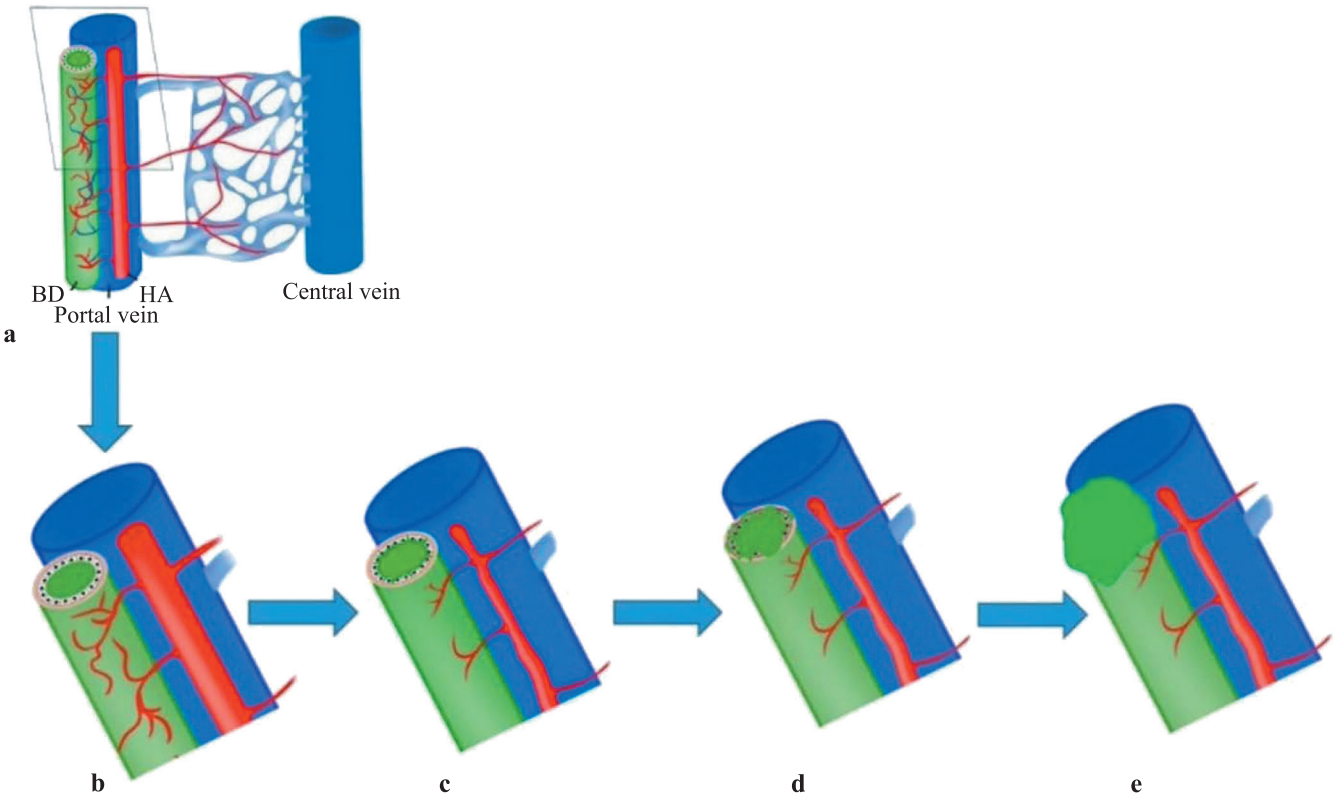

Fig. 7. Schematic influence of ischemia on formation of post-liver transplant biliary complications: a, the bile ducts (BD) are supplied by the hepatic artery (HA), which forms an arterial network around the bile ducts – the peribiliary plexus. The peribiliary plexus drains into the hepatic sinusoids via the periportal plexus The arterial collateral network ruptures after transplantation, leaving the bile ducts at greater risk of ischemia; b, the arterial collateral network is compromised after transplantation due to reperfusion syndrome or arterial stenosis/thrombosis, so the bile ducts are at greater risk of ischemic injury; c, ischemic bile duct injury resulting in epithelial damage due to stenosis, HA thrombosis; d, persistent ischemia progresses and leads to bile duct necrosis; e, formation of a biliary fistula. Ischemia can also lead to formation of bile duct strictures and cholangiogenic abscesses [46]

peritonization of the biliary anastomosis with the round ligament of the left lateral sector graft has been reported (7.6% *vs.* 13.7%).

If CMV infection is detected both before and after LT, it is recommended to prescribe specific antiviral therapy since CMV infection is a proven factor in the development of BCs [63, 98].

CONCLUSION

Biliary complications, known as the Achilles' heel of LT, occur in a quarter of liver recipients. The incidence has increased in recent years due to the increasing number of liver transplant operations worldwide. Living donor LT has a higher rate of BCs and involves more complex scenarios. Endoscopic treatment is the key therapy for most BCs in patients with biliobiliary anastomosis. Ultrasound- and fluoroscopic-guided percutaneous techniques are alternative options for access to bile ducts when endoscopic resolution is ineffective. Treatment of BCs in patients with biliodigestive anastomosis is more complicated due to limitations in endoscopic technology and more often requires invasive, including reconstructive, surgical interventions.

There are a number of directions that need to be developed for more effective treatment and prevention of BCs. It is necessary to pay attention to minimally invasive techniques (including ERCP), especially in the treatment of anastomotic biliary strictures. More functional duodenoscopes are already being developed to enable treatment of complications in the presence of biliodigestive anastomosis. This development has the potential to reduce the risk of relaparotomy in this group of patients. Also, further study of risk factors and their influence on the development of BCs, as well as development of strategies to reduce risk factors will help to prevent BCs, which, in turn, may reduce liver recipient morbidity. Reducing ischemia-reperfusion injury to the graft may also potentially reduce the risk of biliary complications. The development of machine perfusion in the context of cadaveric LT could potentially solve this problem, but further research in this area is required.

The authors declare no conflict of interest.

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DIAGNOSTIC VALUE OF ANTI-HLA ANTIBODY MONITORING IN THE DIAGNOSIS OF IMMUNOLOGICAL COMPLICATIONS FOLLOWING KIDNEY TRANSPLANTATION

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Introduction. Despite improvements in immunosuppressive therapy procedures, immunological complications continue to be a major cause of kidney graft loss. The level of pre-existing and *de novo* synthesized anti-HLA antibodies (AB) has shown high significance in modern diagnosis of graft rejection and assessment of the efficacy of anti-crisis therapy. **Objective:** to analyze the frequency and specificity of pre-existing and *de novo* synthesized (including donor-specific), anti-HLA antibodies, to assess their impact on acute rejection crisis and kidney transplant (KT) outcomes in the early postoperative period. **Materials and methods.** We retrospectively analyzed the treatment outcomes of 637 patients, who received a deceased-donor kidney transplant at Sklifosovsky Research Institute of Emergency Care from 2020 to 2022. Pre-existing and *de novo* synthesized anti-HLA AB, including donor-specific antibodies (DSA), were determined and their impact on the incidence of acute rejection crisis (ARC) in the early postoperative period and on kidney graft function was assessed. **Results.** In non-sensitized patients, the ARC rate was 10.7% (n = 58), primary initial graft function was noted in 354 patients (65.6%), and satisfactory function at discharge was observed in 377 patients (70%). Pre-existing anti-HLA AB was detected in 97 recipients (15.2%); ARC developed in 14 recipients (14.4%) from this group, 51 (52.6%) patients had primary initial function, and 62 (63.9%) exhibited satisfactory function at discharge. *De novo* anti-HLA AB synthesis after transplantation was noted in 70 (11%) patients, ARC in 10 of them (16.7%), 38 (54.3%) had primary function, and 43 (61.4%) had satisfactory function at discharge. DSA synthesis was detected in 10 patients, ARC was diagnosed in 5 (50%) of them, primary initial function and satisfactory function at discharge were noted in 3 (30%) recipients. **Conclusions.** The presence of pre-existing and/or *de novo* anti-HLA AB synthesis after KT under rationally selected immunosuppressive therapy did not statistically significantly affect the early outcomes of graft function. However, DSA synthesis statistically significantly increased the incidence of acute rejection, kidney graft dysfunction and increased the time of recovery of nitrogen excretory function.

Keywords: anti-HLA antibodies, pre-existing anti-HLA antibodies, de novo anti-HLA antibodies, donor-specific anti-HLA antibodies, acute kidney graft rejection.

INTRODUCTION

According to the registry of the Russian Dialysis Society, as of 2020, 60,547 patients with chronic kidney disease stage 5 (stage 5 CKD) had received renal replacement therapy (RRT) in the Russian Federation. This translates into a rate of 414.2 people per million population. RRT dialysis therapy was administered to 50,563 patients (83.5% of all RRT patients); hemodialysis patients made up over 78% of the RRT population, while peritoneal dialysis was received by 4.3% of patients [1]. In 2022, 1,562 kidney transplants were performed in Russia, a 12.9% increase (+178 kidney transplants) over 2021 [2]. Kidney transplantation (KT) is the most desired modality of RRT – it not only increases the life expectancy of patients but also improves their quality of life and offers the best possible medical and social rehabilitation [3–5].

Despite advancements in immunosuppressive therapy protocols, immunological complications remain one of the leading causes of kidney graft loss [6]. About 5–10% of recipients return to RRT within the first year after KT due to graft dysfunction [7].

Renal graft survival is largely determined by the major histocompatibility complex genes and the leukocyte antigens they encode [8]. It is known that the incompatibility of human leucocyte antigens (HLA) in the donor-recipient pair when there is insufficient pro-

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phylactic anti-crisis therapy causes an immune response against the graft, ultimately leading to its destruction. Determination of the level of pre-existing and *de novo* synthesized anti-HLA antibodies (ABs) is of high importance in the diagnosis of graft rejection and evaluation of the effectiveness of anti-rejection therapy. Numerous studies claim that anti-HLA ABs are found in more than 30% of the population and they are formed after blood transfusion, pregnancy or previous transplantation [9, 10].

Donor-specific antibodies can also be formed *de novo*, increasing the risk of antibody-mediated acute and chronic graft rejection and worsening the prognosis of renal allograft (RA) survival [11]. One of the main causes of RA endothelial activation is the binding of circulating anti-HLA donor-specific antibodies (DSAs) to endothelial cells, which contributes to the process of antibody-mediated rejection (AMR). Anti-HLA DSAs are also associated with increased risk of RA rejection and loss [12, 13]. According to various sources, acute AMR occurs in 7% of patients but can be as high as 50% in patients with HLA-incompatible grafts [14].

Currently used immunosuppressants have extremely potent, rejection-blocking effects; however, none of them are antigen-specific [15]. Careful immunological screening before and after transplantation is of paramount importance to minimize the risk of AMR in order to preserve and ensure maximum efficiency of RA function. Currently, many studies have been published on the role of pre-existing and *de novo* synthesized anti-HLA ABs in acute rejection crisis (ARC) after KT, but no such analysis has been performed in our country, which is why we carried out this study.

Objective: to analyze the frequency and specificity of pre-existing and *de novo* synthesized (including donor-specific), anti-HLA ABs, to assess their impact on ARC and KT outcomes in the early postoperative period.

MATERIALS AND METHODS

At the kidney and pancreas transplantation department of Sklifosovsky Research Institute of Emergency Care, 637 single-group deceased-donor kidney transplants were performed in the period from 2020 to 2022.

Inclusion and exclusion criteria

Inclusion criteria: technically successful deceased-donor KT, assessed pre-existing anti-HLA ABs levels (so-called "zero point").

Exclusion criteria: combined transplantation with other solid organs (kidney + liver, kidney + pancreas), living related KT, technically unsuccessful operations (intraoperative graft removal); no "zero point" for anti-HLA ABs.

Recipients

The study group comprised 388 men (60.9%) and 249 women (39.1%), with a median age of 43 [35–53] years. Prior to transplantation, 566 patients (88.8%) received RRT: 434 (68.1%) via hemodialysis, 132 (20.7%) via peritoneal dialysis; 220 (34.5%) recipients had blood group 0 (I), 248 (38.9%) had blood group A (II), 127 (20%) had blood group B (III) and 42 (6.6%) had blood group AB (IV).

Immunological compatibility/incompatibility

The median number of matches (compatibility) for HLA antigens in the donor-recipient pair for class I was 1 [0; 1] antigen (25 [0; 25]%); for class II was 1 [0; 1] antigen (50 [0; 50]%), and the median overall compatibility was 2 [1; 2] antigens (33.3 [16.7; 33.3]%). The median number of class I mismatches, respectively, was 3 [3; 4] antigens (75 [75; 100]%), the median number of class II mismatches was 1 [1; 2] antigen (50 [50; 100]%), and the median total incompatibility by HLA antigens in the donor-recipient pair was 4 [4; 5] antigens (66.7 [66.7; 83.3]%).

Kidney transplant features

Most patients (n = 565, 88.7%) had primary KT, 67 (10.5%) had second transplantation, and 7 (0.8%) had third transplantation.

Immunosuppressive therapy features

In the postoperative period, patients received triple-medication baseline immunosuppressive therapy (IST): tacrolimus was administered as a calcineurin inhibitor in 559 recipients (87.7%) and cyclosporine in 78 (12.3%). As the second component of IST, 612 recipients (96%) received mycophenolate, 19 recipients (3%) were given mTOR inhibitors (everolimus), and 6 patients (1%) were administered azathioprine. Monoclonal antibodies (basiliximab) were used as induction IST in 531 patients (83.3%) and polyclonal antibodies in 66 patients (10.4%), of which 58 patients (9.1%) received antithymocyte immunoglobulin (rabbit) and 8 recipients (1.3%) received antithymocyte equine immunoglobulin. In 40 patients (6.3%), mono- or polyclonal antibodies were not included in the induction IST.

Determination of pre-existing and *de novo* synthesized anti-HLA antibodies

When all patients were placed on the waiting list, anti-HLA class I and class II ABs were determined in venous blood. The study was performed on the Luminex platform, using LABScreen (ONE LAMBDA, USA) and LIFECODES Lifescreen Deluxe (IMMUCOR, USA) kits. With MFI (mean fluorescence intensity) of less than 500 units, anti-HLA ABs were considered negative. *De novo* anti-HLA ABs were defined as anti-HLA ABs that appeared after transplantation or when the level of

pre-existing anti-HLA ABs increased after transplantation by more than 10% of the baseline MFI. *De novo* anti-HLA ABs synthesized in the post-transplant period but not detected in the control study were classified as "transient". *De novo* anti-HLA ABs synthesized after transplantation and detected in the control study were classified as "persistent".

Determination of donor-specific antibodies

The patient's venous blood was tested for HLA antibodies on the Luminex platform using LIFECODES LSA Class I and LIFECODES LSA Class II reagents (IMMUCOR, USA). The anti-HLA ABs specificities detected in the recipient were compared with the donor's HLA typing data – the so-called "virtual cross-match". The match between the identified recipient's ABs and the donor's major histocompatibility complex antigens was considered as the presence of DSAs in the recipient.

Diagnosis of acute rejection

In the presence of one or a combination of several symptoms listed below – decreased diuresis, pain in the kidney graft area, subfebrile body temperature without an infection source, excessive increase in nitrogenous waste (serum creatinine and blood urea) after ruling out other causes of graft dysfunction, changes in ultrasound images over time (appearance of graft edema signs, deterioration of blood supply characteristics), and an increase in the level of *de novo* anti-HLA ABs on the background of or without a decrease in the anti-HLA ABs level. A diagnosis of rejection was made based on a combination of clinical and morphological signs.

Patient allocation into groups

To evaluate the impact of pre-existing and *de novo* synthesized anti-HLA ABs on the incidence of ARC and KT outcomes, patients were divided into the following groups: I_{sens-} and II_{sens+} and $I_{de\ novo-}$ $II_{de\ novo+}$, respectively. In addition, patients were categorized into I_{DSA-} and II_{DSA+} groups based on the presence/absence of DSA. These groups did not differ statistically significantly in terms of the main characteristics (age, sex, body mass index, RRT type and duration, blood group ($p > 0.05$).

Statistical processing methods

Statistical analysis was performed using the programs StatTech v. 2.8.8 (StatTech LLC, Russia) and IBM SPSS Statistics v 24 (IBM, USA). Quantitative parameters were evaluated for conformity to normal distribution using the Shapiro–Wilk test. Quantitative data were described using median and lower and upper quartiles. Categorical data were described using absolute values and percentages. The two groups were compared by quantitative indicator having normal distribution, provided the variance was equal, using Student's t test. The Mann–Whitney U test was used to compare two groups based on a quantitative indicator whose distribution deviated from normal. Patient and graft survival were calculated using the Kaplan–Meier estimate. The 95% confidence interval limits were computed to assess the significance of the odds ratio. Differences were considered statistically significant at $p < 0.05$.

RESULTS

Follow-up periods

Anti-HLA AB levels were monitored during the post-KT in-hospital period, with a median hospital stay of 19 [15–27] days.

Pre-existing anti-HLA antibodies

Pre-existing anti-HLA ABs were detected in 97 recipients (15.2%), including 36 patients after the second transplant surgery and 3 patients after the third. Anti-HLA class I ABs were noted in 68 patients (10.7%), their levels ranged from 505 to 14,444 MFI, with a median level of 1,332 [657–4,093] MFI; anti-HLA class II ABs were noted in 61 patients, their levels ranged from 503 to 14,116 MFI, with a median level of 1,724 [794–7550] MFI; 32 patients had elevated levels of pre-existing HLA class I and class II anti-HLA ABs. During the entire post-transplant follow-up period, no synthesis of anti-HLA ABs was observed in 38 initially sensitized patients.

De novo synthesized anti-HLA antibodies

In the early post-transplant period, *de novo* synthesis of anti-HLA ABs was noted in 70 patients (11%), 37 of whom were not sensitized before surgery. Synthesis of "persistent" ABs was noted in 56 recipients, and "transient" ABs in 14 patients. In 33 patients, *de novo* anti-HLA class I ABs were detected, their levels varied from 504 to 14,729 MFI, with a median level of 2,259 [744–7,672] MFI; *de novo* anti-HLA class II ABs were detected in 58 patients, their levels ranged from 506 to 17,115 MFI, with a median level of 2,983 [917–9,699] MFI; both class I and class II *de novo* anti-HLA ABs were detected in 21 patients. Their detection time ranged from 7 to 46 days; median detection time was 9 [7–13] days.

Donor-specific antibodies

Following identification and comparison with donor antigens, DSAs were detected in 10 recipients: against class I antigens in 2 patients, against class II in 3 patients, and against both classes in 5 patients.

Comparative characteristics of ARC and kidney transplantation outcomes in the study groups are presented in Tables 1, 2, and 3.

Anti-crisis therapy

Seventy-two recipients had ARC at the hospital stage, with 14 of them having been sensitized prior to transplantation, and 10 recipients had *de novo* synthesis of anti-HLA antibodies. Pulse glucocorticoid therapy was

administered to 70 patients as a part of anticrisis therapy: it was used as a part of combined anticrisis therapy in 17 of them, and as monotherapy in 53 patients; its course dose varied from 500 to 1,500 mg, the median was 1000 [1000–1250] mg. Twelve patients had polyclonal antibody infusions as part of a combined anti-crisis therapy, while only two recipients received them as monotherapy; the median number of infusions was 10 [7–10] and the median course dose was 475 [425–3,250] mg. Plasmapheresis was used as part of a combination anti-crisis therapy in the vast majority of cases (n = 12) and as monotherapy in just one recipient. The median number of plasmapheresis sessions was 3.5 [3–4].

DISCUSSION

KT remains the best modality for RRT, providing the longest possible life expectancy, better quality of life and higher socio-medical rehabilitation. The big problem of modern world transplantation is the mismatch between the need and the possibility of providing transplant care for patients with stage 5 CKD. In addition, acute rejection is still widely common in the early post-transplant period, which significantly lowers kidney graft survival. For this reason, many transplantologists recognize the paramount need to advance techniques for diagnosing and treating immunological complications following KT. The importance of identifying pre-existing and *de novo* synthesized anti-HLA ABs, including donor-specific

Table 1

Comparative characteristics of transplant outcomes in $I_{sens–}$ and II_{sens+} groups

Parameter	$I_{sens–}$ (n = 540)	II_{sens+} (n = 97)	P
Immunological complications			
Acute rejection crisis (n (%))	58 (10.7)	14 (14.4)	0.29
Time of rejection onset* (days)	10 [6.17]	8 [4.11]	0.35
Methylprednisolone pulse therapy (n (%))	55 (95)	13 (93)	0.35
Polyclonal antibodies (n (%))	12 (20.7)	2 (14.3)	0.92
Plasma exchange (n (%))	8 (13.8)	5 (35.7)	0.02**
Outcomes			
Primary/delayed function (n (%))	354 (65.6) / 186 (34.4)	51 (52.6) / 46 (47.4)	0.01**
Time to normalization of azotemia (days)	8 [5.13]	9 [4.15]	0.45
Satisfactory KA function at discharge (n (%))	377 (70)	62 (64)	0.31
KA dysfunction (n (%))	142 (26.2)	25 (25.8)	0.96
Non-functioning KA (n (%)) – In-hospital removal of KA (n (%)) – Continuation of RRT at discharge (n (%))	16 (3) 5 (0.9) 11 (2)	8 (8.4) 1 (1) 7 (7.2)	0.01** 0.9 0.004**
In-hospital mortality (n (%))	5 (0.9)	2 (2)	0.32

Note: KA, kidney allograft; RRT, renal replacement therapy. * = Me [25%; 75%]; ** = statistically significant difference (p > 0.05).

Table 2

Comparative characteristics of transplant outcomes in $I_{de\ novo–}$ and $II_{de\ novo+}$ groups

Parameter	$I_{de\ novo–}$ (n = 567)	$II_{de\ novo+}$ (n = 70)	P
Immunological complications			
Acute rejection crisis (n (%))	62 (11)	10 (16.7)	0.43
Time of rejection onset* (days)	10 [7.19]	7 [6; 13]	0.32
Methylprednisolone pulse therapy (n (%))	60 (96.7)	8 (80)	0.83
Polyclonal antibodies (n (%))	10 (16.1)	4 (40)	0.03**
Plasma exchange (n (%))	6 (9.7)	6 (60)	<0.001**
Outcomes			
Primary/delayed function (n (%))	366 (64.6) / 201 (35.4)	38 (54.3) / 32 (45.7)	0.09
Satisfactory KA function at discharge (n (%))	395 (70.4)	42 (60.9)	0.1
Time to normalization of azotemia (days)	8 [5.14]	9 [5.15]	0.28
KA dysfunction at discharge (n (%))	150 (26.7)	21 (30.4)	0.51
Non-functioning KA (n (%)) – In-hospital removal of KA (n (%)) – Continuation of dialysis at discharge (n (%))	16 (2.9) 5 (0.9) 11 (2)	6 (8.7) 1 (1.5) 5 (7.2)	0.01** 0.65 <0.01**
In-hospital mortality (n (%))	6 (1)	1 (1.4)	0.78

Note: KA, kidney allograft.

Table 3

Comparative characteristics of transplant outcomes in I_{DSA-} and II_{DSA+} groups

Parameter	I_{DSA-} (n = 627)	II_{DSA+} (n = 10)	P
Immunological complications			
Acute rejection crisis (n (%))	67 (10.7)	5 (50)	<0.001**
Time of rejection onset* (days)	9 [6.17]	14 [9; 32]	0.27
Methylprednisolone pulse therapy (n (%))	64 (95.5)	4 (80)	0.003**
Polyclonal antibodies (n (%))	14 (21)	0 (0)	0.63
Plasma exchange (n (%))	11 (16.4)	2 (40)	<0.001**
Outcomes			
Primary/delayed function (n (%))	402 (64.1) / 225 (35.9)	3 (30) / 7 (70)	0.03**
Time to normalization of azotemia (days)	8 [5.13]	14.5 [8.21]	0.1
Satisfactory KA function at discharge (n (%))	435 (69.4)	3 (30)	0.006**
KA dysfunction at discharge (n (%))	163 (26)	6 (60)	0.02**
Non-functioning KA (n (%)) – In-hospital removal of KA (n (%)) – Continuation of dialysis at discharge (n (%))	22 (3.5) 6 (1) 16 (2.6)	1 (10) 0 (0) 1 (10)	0.28 0.75 0.15
In-hospital mortality (n (%))	7 (1.1)	0	0.74

Note: KA, kidney allograft.

ABs, in patients undergoing KT has been reported in other countries, but there have been remarkably few of these studies conducted in our country.

According to international experts, over 25% of patients undergoing KT may have elevated levels of pre-existing anti-HLA ABs [16–18]. The donor specificity levels of these ABs are often clinically significant. Our investigation found that 97 recipients (15.2%) of the study group had pre-existing anti-HLA ABs. The sensitized patients had statistically significantly higher frequency of delayed initial KT function compared to patients without pre-existing anti-HLA ABs (47.4% and 34.4%, respectively; p = 0.01). Notably, patients with and without pre-existing anti-HLA antibodies did not differ statistically significantly in terms of ARC frequency and period (14.4% and 10.7%, p = 0.29; 8 [4.11] days and 10 [6.17] days, p = 0.35, respectively). This is probably due to the more frequent use of polyclonal antibodies as part of induction IST in the sensitized patient group. In-hospital kidney graft survival was statistically significantly lower in patients with pre-existing anti-HLA ABs (91.6% and 97%, respectively, p = 0.01), with the incidence of hospital RA removal not significantly different (1% *vs.* 0.9%, p = 0.9), and the incidence of non-functioning RA with patient discharge to continue RRT was significantly higher in II_{sens+} patients (7.2% *vs.* 2% (p = 0.004). In-hospital recipient survival was statistically significantly independent of the presence/absence of pre-existing anti-HLA ABs (98% and 99.1%, respectively, p = 0.32).

Jung et al. reported that the *de novo* synthesized anti-HLA AB rate was 15.5%. Most researchers agree that *de novo* synthesized anti-HLA ABs can dramatically raise the probability of delayed renal graft function, acute rejection crisis, and decreased renal graft survival without reducing recipient survival [19]. Seventy recipients (11%) had *de novo* synthesis of anti-HLA ABs. At the same time, 34% of patients with prior sensitization had *de novo* formation of anti-HLA ABs, compared to 7.4% in the I_{sens-} group (p < 0.001). Moreover, our investigation found that *de novo* antibody formation did not statistically significantly increase the frequency of ARC (16.7% and 11.1% (p = 0.43). Patients in the $I_{de\ novo-}$ and $II_{de\ novo+}$ groups did not significantly vary in terms of incidence of primary/delayed initial RA function. Despite the anti-crisis therapy provided, which, in the $II_{de\ novo+}$ group, significantly more frequently included polyclonal anti-HLA ABs (40% *vs.* 16%, p = 0.03) and plasmapheresis sessions (60% *vs.* 10%, p < 0.001), in-hospital RA survival in this group was statistically significantly lower (91.3% *vs.* 97.1%, p = 0.01). In-hospital survival was statistically significantly lower in patients discharged with a non-functioning graft to continue RRT (7.2% *vs.* 2%, p < 0.01). In-hospital recipient survival was not statistically significantly different between the groups with and without *de novo* synthesis of anti-HLA ABs (98.6% versus 99%, respectively, p = 0.78).

Some researchers estimate that the frequency of donor-specific anti-HLA AB synthesis could reach 10%, while others estimate that it could be as high as 20%. Current theories suggest that DSA increases the incidence of acute rejection and renal graft loss by a statistically significant amount [19, 20]. In our study, the relatively low incidence of *de novo* DSA synthesis (1.6%) was due to the fact that patients awaiting repeat KT were carefully selected for the organ, taking into account immunological compatibility/incompatibility by HLA antigens in the donor-recipient pair, including identification. In addition, induction immunosuppressive therapy by polyclonal antithymocyte ABs was performed for 4–7 days, as well as individual selection of baseline immunosuppressive therapy. Synthesis of donor-specific anti-HLA ABs statistically significantly increased

the frequency of delayed initial graft function (70% *vs.* 35.9%, p = 0.03), acute rejection crisis (50% *vs.* 10.7%, p < 0.001), graft dysfunction at the time of discharge (60% *vs.* 26%, p = 0.02), and it decreased the frequency of satisfactory RA function at recipient discharge (30% *vs.* 69.4%, p = 0.006). Meanwhile, in-hospital survival of RA (90% *vs.* 96.5%, p = 0.28) and recipients (100% *vs.* 98.9%, p = 0.74) were not statistically significantly different between the groups.

CONCLUSION

Identifying patients with increased immunological risk before KT is carried out helps in the rational selection of a donor, the administration of immunosuppressive therapy and, ultimately, the improvement of KT outcomes. Thorough immunological screening for donor-specific ABs and organ organ selection require special consideration.

The authors declare no conflict of interest.

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TRANSJUGULAR INTRAHEPATIC PORTOSYSTEMIC SHUNT WITH GASTRIC VEIN EMBOLIZATION IN LIVER CIRRHOSIS

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Objective: to determine the predictors and risk of recurrent bleeding after implantation of a transjugular intrahepatic portosystemic shunt (TIPS) combined with selective gastric vein embolization in patients with decompensated cirrhosis awaiting liver transplantation (LT). **Materials and methods.** A comparative retrospective study was performed in 54 patients waitlisted for LT between 2017 and 2023, who suffered recurrent variceal hemorrhage after secondary prophylaxis of bleeding prior to inclusion in the study. Demographic, clinical and laboratory parameters, clinical indices, hepatic encephalopathy, severity of ascites, degree of varices, manometric study before and after TIPS implantation with gastric vein embolization, with calculation of portal pressure gradient in patients with (n = 16) and without rebleeding (n = 38), were analyzed. The proportions of patients were compared using the Kaplan–Meier method with determination of the logarithmic test (Log-Rank). Cumulative risks were estimated by means of univariate and multivariate analysis of the Cox proportional hazards model. **Results.** Within 30 weeks from the date of TIPS combined with gastric vein embolization, 16 of 54 patients (29.6%) developed rebleeding. The following risk factors were identified: age, hemoglobin level, white blood cell count, platelet count, creatinine level, severity of ascites, and mean portal pressure gradient after TIPS implantation. It was found that the proportion of patients without bleeding was significantly higher in patients with portal pressure gradient ≤10 mmHg than in patients with this index >10 mmHg (Log Rank = 0.029). The following independent predictors of recurrent hemorrhage were determined: severity of ascites, shunt thrombosis, portal pressure gradient after TIPS implantation, portal pressure gradient after TIPS implantation <30% of the basal level. It has been shown that the risk of recurrent bleeding at portal pressure gradient >10 mmHg progressively increases and reaches maximum values (HR = 1.713) in patients who underwent TIPS combined with gastric vein embolization between 32 and 40 weeks from the time of surgery, while it is absent at portal pressure gradient ≤10 mmHg.

Keywords: liver transplantation, ascites, recurrent variceal bleeding, transjugular intrahepatic portosystemic shunt, portal pressure gradient, risk factors, independent predictors.

INTRODUCTION

The rising number of liver transplants (LT) worldwide, and in Russia in particular, has created a gap between the need for this life-saving operation and the number of donors (donor organs), despite the apparent increase in the activity of donor and transplant coordination centers at a modern stage [1, 2]. Due to the increasing waiting time for LT in patients on the waiting list, progressive decompensation (PD, hereinafter "decompensation") of liver cirrhosis causes a high risk of complications, such as diuretic-resistant ascites, bleeding, and hepatic encephalopathy (HE), increasing to high mortality [3]. Bleeding esophageal varices increases the risk of mortality in patients who are potential candidates for liver transplantation (LT) [4]. After the first bleeding episode, certain patients are at risk of recurrent bleeding (RB) developing early or late, thereby increasing the overall waitlist mortality [5, 6]. The International Committee on the Management of Patients with Portal Hypertension recommend two RB prophylaxis strategies, implemented by first-line therapy, and in case of failure, by second-line therapy [7]. First-line therapy consists of a combination of interventional procedure – endoscopic variceal ligation (EVL) with administration of non-selective beta-blockers (NSBB). The second-line therapy involves implantation of a transjugular intrahepatic portosystemic shunt (TIPS) [7].

Currently, polytetrafluoroethylene (PTFE)-coated TIPS is a well-established intervention for the treatment of complications of portal hypertension (PH) [7, 11–13].

Current guidelines recommend a further development of this procedure for this purpose – a combination of TIPS procedure and extrahepatic collateral vessel chemoembolization (TIPS + GVE) in order to control variceal bleeding and reduce the risk of rebleeding [7, 8, 11, 14]. Implementation of both variants of invasive in-

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terventions (TIPS or TIPS + GVE) has brought attention to the issue of striking a balance between reduction in RB and development of HE, which are linked to hemodynamic changes brought on by these interventional procedures [8, 9]. Portal pressure gradient (PPG) dynamics is the most important characteristic of hemodynamic response after TIPS or TIPS + GVE [8–10].

Decreased PPG due to TIPS procedure raises two big problems that need to be solved: how to effectively prevent rebleeding and how to avoid an increase in the grade of overt HE caused by excessive shunting of portal blood flow [8–10].

A comparative assessment of the effect of a combination of procedures (TIPS + GVE and TIPS) on RB prevention and HE development has very contradictory data and calls for further studies [8–10].

Previous studies have found that the target PPG level after a TIPS procedure is <12 mmHg [15]. Another target is a >50% PPG decrease from baseline before shunt placement [16]. Both indicators ensure effective prevention of RB and other complications of PH [15–17], being a Baveno VII recommendation for the management of patients with PH [7].

The definition of PPG thresholds was derived from careful preliminary studies showing that RB and ascites almost exclusively develop in patients with a PPG of at least 12 mmHg after stent implantation [9, 15–18].

It is important to note that these thresholds of PPG decrease to the target level were achieved prior to coated stents being used in clinical settings [15, 18], which highlights the need to improve them [10]. Specifically, the risk of severe HE is quite high with coated TIPS, even with the current standards, limiting their use in clinical practice [10, 19, 20].

Therefore, it is crucial to determine the threshold of PPG decrease after coated TIPS implantation in order to determine the risks of RB and overt HE [15–17]. Since PPG may, according to Wang et al. [21], increase after GVE, this should be considered when implanting a stent and achieving target gradient values.

Objective: to determine the predictors and risk of RB after PTFE-covered TIPS implantation combined with selective GVE in patients with decompensated cirrhosis awaiting LT.

MATERIALS AND METHODS

A comparative retrospective study was performed in 54 patients with decompensated cirrhosis who were on the LT waitlist between 2017 and 2023. Prior to inclusion in the study, all patients in this group developed recurrent variceal rebleeding after secondary prophylaxis of bleeding through a NSBB + EVL combination.

After approval by the local ethics committee at the Center for Surgery and Donation Coordination (CSDC), Rostov Regional Clinical Hospital, the patients were included in a study of the efficacy and safety of PTFE-covered TIPS procedure combined with GVE.

Inclusion criteria: patients of either sex aged 18 to 75 years, cirrhosis of any etiology: virus-related (HBV- or HCV-), alcohol-related, or cirrhosis of mixed etiology (virus-related and alcohol-related), RB after combination (EVL + NSBB) therapy, complete abstinence in patients with alcohol-related cirrhosis for at least 3 months prior to inclusion in the study (confirmed by narcologists), CTP classes B and C, indications for TIPS procedure [7, 11, 13], availability of a complete electronic database with demographic, clinical, laboratory parameters and instrumental studies, presence of complete hemodynamic parameters before and after PTFE-covered TIPS implantation and GVE.

Exclusion criteria: Hepatocellular carcinoma or any other tumors, severe liver failure, heart failure, severe renal failure, recurrent or persistent overt HE despite adequate therapy, infectious diseases, sepsis; presence of contraindications for TIPS procedure, presence of CTP score >15, MELD-Na score >27, previous surgical shunt or LT, non-cirrhotic (idiopathic) portal hypertension (PH), sinusoidal obstruction syndrome or Budd–Chiari syndrome, portal vein thrombosis or cavernous transformation, pregnancy or lactation.

A regularly updated electronic database of patients with demographic, clinical, and laboratory information, who were on the LT waitlist of the Center for Surgery and Donation Coordination (CSDC), Rostov Regional Clinical Hospital, served as the foundation for the analysis that followed. At CSDC, patients were managed by specialists. When patients were included in the study, they were examined, including undergoing laboratory and instrumental tests (full blood count and biochemical tests, hemostasis indicators, MELD-Na score and CTP class). The frequency of these tests was determined by patient condition. When the patient's condition was stable, repeated examinations were performed once every three months; and abdominal ultrasound once every 6 months. For unstable patients included in the study, the need for laboratory and instrumental studies was determined by the presence of indications.

Esophagogastroduodenoscopy (EGDS) was used to screen all patients for varices with high risk of bleeding (medium and large-sized varices requiring bleeding prophylaxis) in accordance with the guidelines of the Baveno VI Consensus Workshop [22] and the World Gastroenterology Association (WGO) [23].

The International Club of Ascites (ICA) criteria were used to grade the severity of diuretic-responsive and diuretic-resistant ascites [24]. In addition to the ICA criteria [24], the Cirrhotic Ascites Severity (CIRAS) scale [25] was used to characterize diuretic-resistant ascites. When patient CIRAS score was 5–6, diuretic-resistant ascites was deemed to be the definitive diagnosis.

Hepatic encephalopathy (HE) was graded according to the modified West Haven criteria recommended by the European Association for the Study of the Liver (EASL) and the American Association for the Study of Liver Diseases (AASLD) [26].

Mean arterial pressure (mAP) was determined by the formula: mAP = (DP) + $^1/_3$(SP – DP), where SP stands for systolic pressure, and DP for diastolic pressure [27].

Patients were given diuretics; 15 patients with diuretic-resistant ascites had paracentesis (7 patients had it once, while 8 had it repeatedly, from 2 to 5). In compliance with recognized expert standards, antiviral medication with nucleoside analogs or a combination of direct-acting antivirals was given if HBV and HCV-associated cirrhosis was diagnosed [26].

Esophageal manometry (EM) was performed on all patients before PTFE-covered TIPS procedure, but after GVE and stent implantation. For this purpose, the J-end of a standard angiographic guidewire was placed in the inferior vena cava (IVC) slightly above the hepatic vein (HV) orifice via transjugular access. EM was performed using a pressure transducer balloon catheter (Edwards Lifesciences, USA) at the end. Immediately after catheter placement into the portal vein (PV), baseline (basal) portal vein pressure (PVP) was measured. After catheter placement in the IVC, baseline basal inferior vena cava pressure (IVCP) was measured. Portal pressure gradient (PPG) was measured by calculating the difference between PVP and IVCP.

TIPS procedure was performed in accordance with the guidelines for the management of patients with decompensated cirrhosis complicated by PH [11]. The shunt was implanted using a Flexor Check-Flo introducer and Rösch curved catheter included in the RUPS-100 instrument set (Cook Medical®, USA) under local anesthesia with additional intravenous sedation with analgesics. After puncture of the right internal jugular vein, under fluoroscopic control a standard angiographic guidewire was advanced through the superior vena cava (SVC) and atrial sinus into the IVC, placing its J-shaped end at a level slightly above the HV orifice. The Flexor Check-Flo introducer with a curved Rösch catheter was guided through the angiographic guidewire, placing them in the right HV closer to its ostium.

The surgical procedure involved creating a tunnel (intrahepatic conduit) running from the right HV to the PV right branch or bifurcation using a Rösch-Uchida needle and a balloon advanced over a guidewire. After balloon retrieval, a PTFE-covered stent graft (Hanarostent® Hepatico (M.I. Tech®) model, 8 or 10 mm in diameter), was implanted through the guidewire. In the next surgical step, the catheter was placed in the splenic vein close to the splenic hilum to perform direct vein portography, which allowed to visualize the mouths of the inflow pathways to the esophageal-gastric varices. Subsequent selective catheterization of the left, posterior and short gastric veins with embolization of each of them was performed. For this purpose, we used MReye® (Cook®) embolization coils, which have high thrombogenicity due to numerous long fibers. The number and size of the coils used were determined by the peculiarities of angioarchitecture, diameter and branches of the inflow pathways and varied from 0 to 14. After vein embolization, PVRembo and IVCPembo were immediately examined and PPGembo was calculated. The surgical intervention was completed by control phlebos shuntography.

The IBM SPSS Statistics software package (version 23) was then used to do a statistical analysis on the collected data. The Kolmogorov–Smirnov test and the Lilliefors significance threshold were used to assess the type of distribution of the obtained variables of the analyzed samples. The arithmetic mean (M) and standard deviation (SD) were computed if it was discovered that the variables had a normal distribution. Using a significance threshold of $p < 0.05$, the Student's t-test was used to assess the significance of differences between the compared values. In the event that it was discovered that the variables did not have a normal distribution, the interquartile range (IQR, the interval between the 25th and 75th percentiles) was used to calculate the median (Me). To determine the significance of differences in pairwise comparisons of dependent variables, the Wilcoxon signed-rank test recommended in nonparametric analysis, was used. Pearson's chi-squared test was used to compare independent variables. For a small sample, the variables were compared using the Mann–Whitney U test. Analysis of variance was carried out through ANOVA test. Conjugacy tables were used to analyze qualitative parameters (frequencies of variables and their percentages); for small samples, Fisher's exact test was used to assess the significance of the relationship between two variables.

The Kaplan–Meier method was used to compare the proportions of patients in different groups. The significance of differences between the compared curves (patient proportions) was determined by calculating the log-rank [Log-Rank (Mantel-Cox)].

Comparative assessment of cumulative accumulated risks in the groups was performed using the mathematical model of proportional risks (Cox regression) in univariate and multivariate analysis. The risk of occurrence of the tested event (HR) was calculated and the 95% confidence interval (CI) for this indicator was determined. The quality of the model used was determined by estimating the maximum likelihood (log-likelihood, –2LL). The condition of multivariate Cox proportional hazards regression analysis (absence of linear relationship between independent variables, which creates redundancy in the model) was verified by constructing a correlation matrix.

RESULTS

Patients included in the study, from the date of TIPS procedure combined with GVE up to 30 weeks of follow-up (n = 54), were divided into two groups. The first group consisted of those who developed RB after TIPS + GVE (n = 16, 29.6%), and the second group consisted of patients who had no RB after this combined surgical intervention (n = 38, 70.4%).

Demographic, clinical, laboratory parameters, as well as MELD-Na and CTP scores in the RB and non-RB group after the TIPS procedure combined with GVE are presented in Table 1.

Table 1 shows that patient age, hemoglobin, leukocyte level, platelet count, creatinine level, ascites severity, and mean PPG following TIPS surgery were significantly different between the compared groups, with the RB group having higher values than the non-RB group.

We also assessed the reduction in PPG after TIPS procedure as a percentage of its basal value (before shunt placement).In the RB group, PPG decreased by less than 30% of its basal value in 13 out of 16 patients (81.25%), but in the non-RB group, PPG decreased by 55.3% in 21 out of 38 patients (p = 0.04). Shunt dysfunction (shunt thrombosis) occurred in 11 out of 16 patients (68.75%) in the RB group, and in 3 out of 38 patients (7.89%) in the non-RB group (p = 0.02).

We compared the frequency of RB in the two groups of patients differing in PPG value.

The first group consisted of patients whose PPG was ≤10 mmHg (n = 15), and the second group consisted of patients with PPG >10 mmHg (n = 7). Ten of the fifteen patients (66.7%) in the first group and six of the seven patients (85.7%) in the second group both experienced RB, difference between groups (p = 0.047).

Using the Kaplan–Meier method, it was established that the proportion of patients without re-bleeding was significantly higher in the group of patients with PPG ≤10 mmHg than in the group with PPG >10 mmHg (Log Rank = 0.029) (Fig. 1).

We used survival analysis to predict the risk of recurrent hemorrhage for patients who underwent TIPS + GVE, while awaiting LT. Biomedical research uses this approach to predict mortality, disease recurrence, recovery, or any other outcomes relative to the time of their occurrence [29]. The influence of independent variables (predictors) on RB risk was investigated using a mathematical Cox proportional hazards model with calculation of the risk of an adverse event (Hazard Risk; HR) and determination of the 95% CI.

For this purpose, we used univariate and multivariate analysis of the mathematical Cox proportional hazards model (Table 2).

When univariate analysis was applied, a model with one independent variable was created with calculation of the hazard ratio (HR), confidence interval (CI) and assessment of the significance of the effect on the develop-

Table 1

Comparative characteristics of patients with and without rebleeding after TIPS procedure in combination with gastric vein embolization (normal distribution and non-normal distribution)

Indicator	RB (n = 16), M ± SD	No RB (n = 38), M ± SD	p-value
Normal distribution (M ± SD)			
Age	55.31 ± 7.26	50.13 ± 10.8	**0.046**
Hemoglobin (g/L)	83.11 ± 19.21	115.78 ± 17.21	**0.038**
White blood cells ($\times 10^9$/L)	3.53 ± 1.35	4.75 ± 1.83	**0.021**
Plasma albumin (g/L)	29.94 ± 3.28	30.66 ± 3.08	0.46
Creatinine (μmol/L)	117.13 ± 20.04	105.42 ± 11.02	**0.042**
INR	1.91 ± 0.25	1.92 ± 0.46	0.91
MELD-Na (points)	21.59 ± 3.13	20.71 ± 2.67	0.31
mAP (mmHg)	90.13 ± 9.98	88.32 ± 10.41	0.57
PPG basal (mm Hg)	25.94 ± 4.14	24.87 ± 2.97	0.64
PPG after embolization (mmHg)	26.11 ± 3.86	26.07 ± 1.14	0.57
PPG after TIPS (mmHg)	10.93 ± 0.76	8.02 ± 0.69	**0.04**
Non-normal distribution (Me; IQR)			
Platelets ($\times 10^9$/L)	75.0 (54.0–95.0)	105.00 (74.75–141.75)	**0.02**
Bilirubin (μmol/L)	68.0 (56.25–86.0)	76.0 (64.5–79.5)	0.36
Na (mmol/L)	130.5 (130.0–137.0)	131.0 (129.75–132.0)	0.56
CTP (points)	10.5 (7.0–14.0)	10.5 (8.0–13.25)	0.70
Ascites grade	2.0 (1.0–2.75)	3.0 (2.00–4.0)	**0.02**
HE grade (points)	2.0 (1.0–3.0)	2.0 (1.75–2.0)	0.71

Note: RB, recurrent bleeding; INR, International normalized ratio; MELD-Na, Model for End-Stage Liver Disease-Sodium; CTP, Child–Turcotte–Pugh; Na, sodium; HE, hepatic encephalopathy; mAP, mean arterial pressure; PPG, portal pressure gradient.

ment of an adverse event (rebleeding) for each predictor. All independent variables (predictors) that significantly influence the development of RB in univariate analysis, are presented in the first part of Table 2.

As can be seen from Table 2, in the univariate analysis of the mathematical Cox proportional hazards model, independent variables that significantly influence the development of RB were identified: ascites severity (gra-

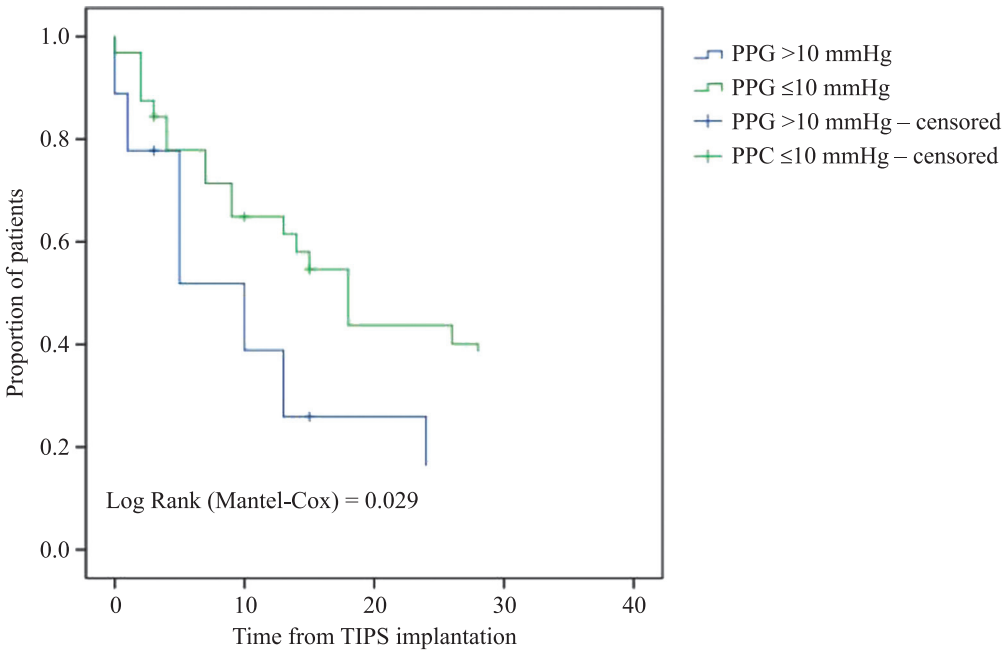

Fig. 1. Proportion of patients without bleeding and with rebleeding after TIPS procedure combined with gastric vein embolization, depending on PPG (Kaplan–Meier method with Log-Rank test)

Table 2

Univariate and multivariate analysis of predictors associated with recurrent bleeding after TIPS procedure combined with gastric vein embolization

Variables	Univariate analysis		Multivariate analysis	
	HR (CI)	p-value	HR (CI)	p-value
Age	1.034 (0.972–1.099)	0.293	–	–
Platelets ($\times 10^9$/L)	0.985 (0.970–1.00)	0.054	–	–
White blood cells ($\times 10^9$/L)	0.696 (0.480–1.010)	0.057	–	–
Plasma albumin (g/L)	0.858 (0.724–1.016)	0.076	–	–
INR	1.214 (0.393–3.749)	0.736	–	–
Bilirubin (μmol/L)	0.999 (0.986–1.011)	0.830	–	–
Creatinine (μmol/L)	1.021 (0.992–1.050)	0.151		
Na (mmol/L)	1.091 (0.988–1.205)	0.363	–	–
Hemoglobin (g/L)	1.011 (0.954–1.151)	0.352	–	–
MELD-Na (points)	1.072 (0.899–1.279)	0.439	–	–
CTP (points)	0.964 (0.812–1.144)	0.673	–	–
Ascites grade	0.479 (0.284–0.807)	**0.006**	0.591 (0.412–0.848)	**0.004**
HE grade (points)	1.137 (0.654–1.974)	0.650	–	–
mAP (mmHg)	1.005 (0.958–1.055)	0.829	–	–
Shunt thrombosis	1.239 (0.945–1.350)	**0.035**	1.003 (0.967–1.367)	**0.041**
PPG basal (mmHg)	1.129 (1.015–1.522)	0.181	1.638 (0.645–4.163)	0.299
PPG after embolization (mmHg)	0.563 (0.312–0.789	0.129	0.811 (0.391–1.684)	0.575
PPG after TIPS (mmHg; cat*)	1.153 (0.997–1.452)	**0.011**	1.168 (0.989–1.435	**0.023**
PPG after TIPS <30% of basal level (mmHg)	1.012 (0.961–1.097)	**0.035**	1.009 (0.834–1.069)	**0.043**

Note: * – variable including two HVPG categories: ≤10 and >10 mmHg. HR, hazard ratio; MELD-Na, Model for End-Stage Liver Disease-Sodium; INR, International normalized ratio; CTP, Child–Turcotte–Pugh; Na, sodium; HE, hepatic encephalopathy; mAP, mean arterial pressure; HVPG, hepatic venous pressure gradient; PPG, portal pressure gradient.

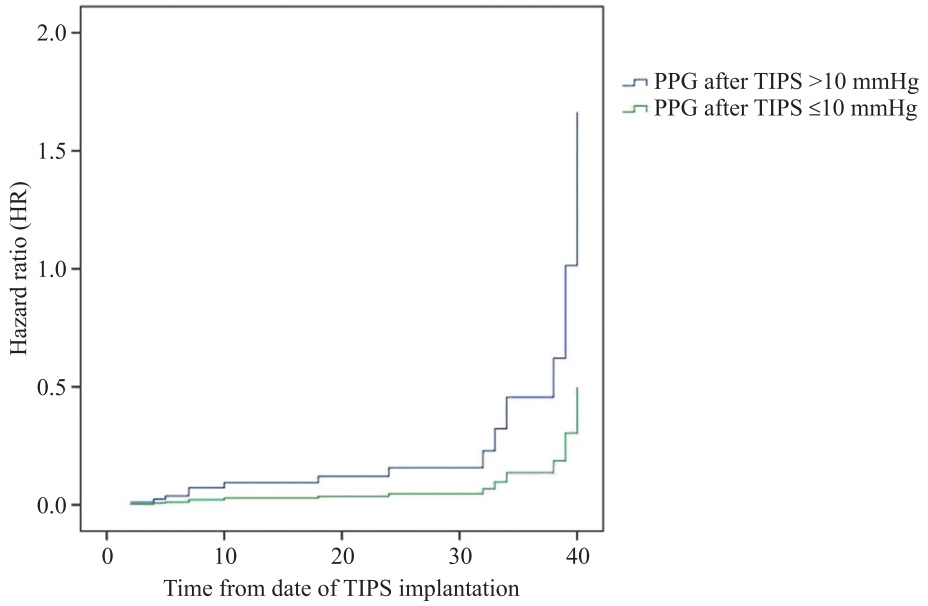

Fig. 2. Hazard ratio (HR) for rebleeding as a function of time and categorical variable PPG after TIPS procedure (≤10 mmHg; >10 mmHg)

de), shunt thrombosis, PPG (cat.) after TIPS, PPG after TIPS <30% of basal level (mmHg).

Multivariate analysis involved the creation of a model designed to assess the independent contribution of several predictors simultaneously, while determining the significance of their influence on RB. The second part of Table 2 shows the effect of all simultaneously acting significant predictors on RB development in the multivariate analysis. This analysis was performed by forced-entry (Enter) method, in which all variables are simultaneously entered into the model. The multivariate analysis model included all statistically significant predictors identified by the univariate analysis (considering each predictor separately), as well as known risk factors for RB, regardless of their influence in the univariate analysis, which is an acceptable technique for building this regression model [29, 30].

As shown in Table 2, a hazard ratio (HR) >1.0 was significant for the presence of shunt thrombosis, post-TIPS (cat.) PPG, and post-TIPS PPG <30% of basal level (mmHg), which allows us to consider these factors as having an independent influence on RB risk.

HR <1 was significant for the independent variable – ascites severity (grade). For HR <1, the influence of these factors is associated with increased survival time, i.e., a factor reducing the RB risk [29, 30].

The quality of our chosen model of multivariate Cox proportional hazards regression analysis was confirmed by estimation of the maximum likelihood indicator (log-likelihood or –2LL). In comparison with the base model (Block 0), the value of –2LL was 99.924; after introducing independent variables (predictors) into the model, –2LL decreased (76.657, Pearson's Chi-square = 23.454) at a significance level of 0.005. This analysis allows us to reject the null hypothesis, which in fact means that the predictive ability of the multivariate Cox proportional hazards regression analysis model is improved when independent predictors are included.

We constructed a correlation matrix to test the condition (no linear relationship between independent variables, which creates redundancy in the multivariate Cox proportional hazards regression analysis model). The correlations found were very weak (0.002 to 0.197), weak (0.198 to 0.395) and moderate (0.396 to 0.510), which does not negatively affect application of the model [29].

In multivariate analysis, we plotted the hazard ratio (HR) of RB for different values of the categorical variable PPG (≤10 mmHg; >10 mmHg) (Fig. 2).

As can be seen from Fig. 2, RB risk at PPG >10 mmHg progressively increases and reaches maximum values (HR = 1.713) in patients who underwent TIPS + GVE at 32 to 40 weeks from the time of surgery, while it is absent at PPG ≤10 mmHg, reaching HR = 0.517 within the same timeframe from the time of surgery.

DISCUSSION

We have shown that in patients who underwent TIPS procedure combined with gastric vein embolization (second-line therapy), due to the failure of previous first-line therapy (EVL + NSBB), 16 of 54 patients (29.6%) developed RB within up to 30 weeks of follow-up after surgery.

Zhao et al. [8] analyzed the incidence of post-TIPS rebleeding combined with GVE. The authors showed that 17.6% of patients included in the study developed RB during a mean 32.5-month follow-up period. The incidence of RB in the TIPS + GVE group was signifi-

cantly lower than in the TIPS group (17.6% and 23.2%, respectively).

A meta-analysis was conducted by a group of researchers to compare the incidence of RB, shunt dysfunction, and other outcomes between the TIPS and TIPS + GVE patient groups [31]. The TIPS + GVE combination was found to have a significantly lower incidence of RB compared to the TIPS group.

However, several studies have found no significant differences in the incidence of RB after these two types of surgery were obtained [32–34].

The higher incidence of RB after TIPS + GVE surgery in our study when compared with other reports is down to several factors. First, the success of RB prevention after TIPS or TIPS + GVE is linked to the PPG level after shunt placement. Bosch showed that to prevent RB after stent implantation, PPG level should be reduced to <12 mmHg (or 50% or more of the PPG level before stent implantation). Controlled stent dilation is recommended until the PPG level is ≤12 mmHg. At the same time, PPG decrease to <10 mmHg causes a significantly higher incidence of HE and acute liver injury [9].

The experience of foreign studies [9, 15] and our results show that, in practice, achieving the target level of PPG decrease (<12 mmHg) in many cases causes significant difficulties faced by the surgeon that is implanting TIPS. This is particularly the case when it comes to performing the stent implantation procedure with controlled dilation until the level of effective PPG decrease is achieved. The narrow therapeutic window for achieving the target PPG level is evidenced by data from a study by Bosch et al. [9], who showed the existing problems on the following example: when the stent is dilated up to 8 mm, PPG is reduced to 13 mmHg, and when the stent is dilated up to 10 mm, PPG decrease can be 6–7 mmHg. It is known that RB after TIPS or TIPS + GVE procedure develops when the target PPG is not reached after stent placement [9, 32, 33].

In our study, we found that PPG >10 mmHg after TIPS + GVE surgery increases the incidence of RB, and being a significant independent predictor, increases the risk of RB. Studies have shown, using multivariate Cox proportional hazards regression model analysis that PPG after TIPS + GVE is an independent predictor of RB [32, 33]. A 1 mm increase in PPG increases the risk of developing RB by 9% [33]. In our study, in multivariate Cox regression analysis, the risk of RB was even higher than in the cited study if PPG after TIPS + GVE was >10 mmHg. Thus, a 1 mm increase in PPG was associated with a 16.8% increase in RB risk.

Second, the increased incidence of RB after the TIPS + GVE procedure may be due to the development of more frequent shunt dysfunction (thrombosis) in our study. Shunt dysfunction occurred in 14 out of 54 patients in the TIPS + GVE group, accounting for 25.9% of cases. Jahangiri et al. [35] showed that primary TIPS thrombosis occurred in 17 cases (9.8%) within up to 24 months of follow-up after shunt surgery, and after 5 years, shunt dysfunction was determined at 21.8%. The annual calculated incidence of thrombosis was 38.7 per 1,000 person/year (95% CI, 19.3–77.3). The risk of shunt thrombosis was found to be related to post-TIPS PPG value. Thus, PPG <5 mmHg, 5–8 mmHg, and >8 mmHg had a 4.3%, 6.4%, and 17.7% risk of shunt thrombosis, respectively.

In our study, shunt dysfunction (shunt thrombosis) occurred in 11 (68.75%) out of 16 patients with RB developing after TIPS + GVE, and in 3 (7.89%) out of 38 in patients without RB, which was significantly lower than in the compared group ($p = 0.02$). In this regard, it is appropriate to cite the results obtained by Rosenqvist et al. [36], who showed that post-TIPS recurrent bleeding was associated with shunt thrombosis on one hand and with post-TIPS PPG on the other hand. The authors concluded that a PPG ≥5 mmHg after TIPS procedure is associated with increased risk of RB, and risk of shunt dysfunction. Another study showed that a post-TIPS PPG of 8.5 mmHg is significant for the development of shunt thrombosis [37].

In addition, our univariate and multivariate analysis of the Cox proportional hazards regression model established that shunt thrombosis is an independent predictor of RB after TIPS + GVE. Jahangiri et al. [35] came to similar conclusions, demonstrating that a 1 mmHg increase in post-TIPS PPG causes a 14% risk of thrombosis ($HR = 1.14$, $p = 0.023$).

The second independent predictor of RB risk after TIPS + GVE that we identified in both univariate and multivariate studies was the severity of ascites. We believe that ascites progression is a consequence of changes in portal hemodynamics and, therefore, may reduce the likelihood of developing RB, because the hazard ratio (HR) for this independent variable was <1. We reference a study by Liu et al. [38] that indicated that patients with ascites have a lower predictive value of hepatic venous pressure gradient (HVPG), an independent predictor of early rebleeding in the absence of ascites. This finding lends weight to our conclusion.

Insufficient decrease in PPG after shunt implantation relative to its basal level (<30%) is another significant predictor of RB after TIPS + GVE in our multivariate analysis.

Using a multivariate Cox model, Biecker et al [39] showed that post-TIPS PPG is an independent predictor of recurrent bleeding. Patients with a <30% post-TIPS PPG decrease were at the highest risk for rebleeding, and, on the contrary, patients with a >60% PPG decrease rarely suffered from rebleeding.

CONCLUSION

Within up to 30 weeks of follow-up after a TIPS + GVE procedure, RB develops in 29.6% of patients who

have been waiting for LT for a long time due to lack of donor organ.

Ascites severity (grade), presence of shunt thrombosis, post-TIPS (cat.) PPG, and post-TIPS PPG of <30% of baseline level (mmHg) are independent significant predictors of RB.

Patients who underwent TIPS + GVE treatment had a progressive increase in risk of developing RB at PPG >10 mmHg, which peaked 32–40 weeks after surgery. At HVPG ≤10 mmHg, however, there is no risk of developing RB.

The authors declare no conflict of interest.

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EARLY AND LONG-TERM OUTCOMES OF DECEASED-DONOR KIDNEY TRANSPLANT IN RECIPIENTS 70 YEARS OF AGE AND OLDER

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Introduction. The high prevalence of chronic kidney disease (CKD) has a negative impact on the length and quality of life of patients, especially in the older age group. Renal replacement therapy is required when the disease progresses to end-stage renal failure. In elderly patients with comorbidities, dialysis therapy has its own peculiarities and challenges, often prolonging life for a short period. The increase in the number of patients aged ≥70 years requesting to be placed in the kidney transplant waitlist (KTWL) at Sklifosovsky Research Institute of Emergency Care has led to the need to evaluate kidney transplant (KT) outcomes in this patient cohort. **Objective.** To analyze the early and long-term outcomes of deceased-donor KT in recipients aged ≥70 years. **Materials and methods.** The retrospective study included 23 kidney recipients aged ≥70 years who underwent a deceased-donor KT in the period from 2014 to 2023 at the Kidney and Pancreas Transplantation Department, Sklifosovsky Research Institute of Emergency Care. Recipient survival was computed using the Kaplan–Meier estimate. **Results.** Sixteen recipients (69.6%) had primary function and 7 (30.4%) had delayed function. Nineteen recipients (82.6%) showed a drop in blood creatinine below 200 μmol/L after KT. Hospital, 1- and 3-year survival were 96% (n = 22), 84.8% [95% CI 72–95] and 79% [95% CI 65–92], respectively; 1- and 3-year graft survival were 84.8 [95% CI 72–95] and 73% [95% CI 59–87], respectively. **Conclusion.** KT for patients aged ≥70 is a feasible treatment option for CKD stage 5.

Keywords: kidney transplantation, kidney recipients aged ≥70, recipient survival, kidney graft survival, kidney transplant outcomes.

INTRODUCTION

Over the past 20 years, there has been an increase in the number of patients aged ≥70 years with stage 4–5 chronic kidney disease (CKD). According to the 2019 World Health Organization report, kidney diseases have become one of the world's top 10 leading causes of death, rising from 18th to the 10th position over a nine-year period, with mortality increasing from 813,000 to 1.3 million [1, 2]. The rise in CKD mortality is most common among individuals aged ≥70 years [2]. Patients can now receive renal replacement therapy (RRT) and save their lives all thanks to improved CKD detection, higher-quality medical care, and the establishment of new dialysis facilities nationwide. Kidney transplantation (KT) is known to be associated with lower mortality and higher quality of life compared to dialysis-based RRT. It is also more cost-effective [3].

In elderly patients, the choice between dialysis and KT may be difficult due to possible adverse outcomes and the heterogeneity of this group in terms of associated geriatric syndromes [4, 5]. Over the last decade, we have seen a rise in the number of potential recipients who are 70 years of age or older in our waiting list. The world is showing a similar trend. In Europe, for instance, the average age of kidney recipients has increased by 10 years over the past two decades, while the proportion of patients aged 65–74 years on the waiting list in the United States rose from 2% in the 1990s to over 10% in 2012 [6]. The number of KTs done on the elderly is increasing because of the aging population [7].

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There is currently no consensus among specialists regarding early and long-term outcomes of KT in patients of this age group, and there are still questions about whether transplantation is necessary when there is comorbid pathology present, which increases the risk of an adverse outcome. A meta-analysis by Artiles et al. revealed lower 5-year survival rates for allogeneic kidney transplant (AKT) recipients aged ≥70 years at any point during the postoperative period [8].

Based on an analysis of the outcomes of 10,651 KTs performed in Australia and New Zealand from 2000 to 2015, of which 279 (2.6%) were KTs to elderly adults (older than 70 years), Doucet et al. reported a lower 5-year survival for age-matched recipients. However, the authors noted that this cohort of patients received AKT predominantly from elderly donors and expanded criteria donors [9]. Greg A Knoll stated in a publication that despite lower survival with KT in the group of patients over 65 years of age when compared with survival in the 30–49-year-old recipient group (61% vs. 75%, respectively), older kidney recipients had survival rates 40–60% higher than those of patients who received dialysis-based RRT [10]. Similar data have been reported by nephrologists from Spain [11].

In our country, there are reports of rare cases of KT in patients older than 70 years old. Therefore, it was decided to evaluate KT outcomes in patients of this age group at the Sklifosovsky Research Institute of Emergency Care.

Objective: to analyze the early and long-term outcomes of deceased-donor KT in recipients aged ≥70 years.

MATERIALS AND METHODS

The study is based on a retrospective review of the outcomes of 23 KT procedures performed at Sklifosovsky Research Institute of Emergency Care from 2014 to 2023 in recipients aged ≥70 years. Inclusion criteria were primary KT from a deceased donor and recipient age 70 years and above. Exclusion criteria were recipient age less than 70 years, KT from a living related donor, KT combined with other organs. The patients were followed up from the moment of KT until graft loss or recipient death.

Study

To assess recipient and AKT survival rates, we used data from the medical records and case histories of patients at the kidney transplant of Sklifosovsky Research Institute of Emergency Care, and outpatient charts of AKT recipients at the Moscow Applied Research Center of Transplanted Kidney Nephrology and Pathology.

Recipients

The study group consisted of 23 kidney recipients. The median age was 72 [70–77] years. Gender distribution: 13 (57%) men and 10 (43%) women. The diseases that led to stage 5 CKD were chronic glomerulonephritis (9, 39.1%), hypertensive nephroangiosclerosis (7, 30.4%), chronic pyelonephritis (3, 13%), polycystic disease (2, 8.7%), urolithiasis (1, 4.4%), and type 2 diabetes mellitus (1 4.4%). Most patients (20 people, 87%) were receiving RRT dialysis at the time of KT, of whom 17 patients (73.9%) through dialysis, three recipients (13%) through hemodialysis, and 3 (13%) patients with a glomerular filtration rate (GFR) <15 mL/min were scheduled to commence dialysis therapy.

Donors

Donor median age was 60 [48–68] years. Gender distribution: 12 (52%) men and 11 (48%) women. All KT were from donors with confirmed brain death resulting from acute stroke (18, 78.3%) and head injury (5, 22%). Donors' median blood creatinine and urea levels at the time of graft procurement were 101.6 [53–214] μmol/L and 7.7 [2.7–14.4] mmol/L, respectively. Bacteriological examination of donor kidney perfusate was negative at all KTs.

Transplantation

AKT was allocated to a specific KTWL patient, determined by the Moscow Coordinating Centre for Organ Donation, taking into account blood group, number of matches for A, B, Dr antigens of the major histocompatibility complex in the donor-recipient pair and negative lymphocytotoxic test. There were mismatches for 5 HLA antigens in 10 donor-recipient pairs (43%), for 4 HLA antigens in 9 pairs (39%), and for 3 HLA antigens in 3 pairs (13%).

KT was performed according to the standard technique: the AKT was placed in the iliac region, its vessels were anastomosed with the recipient's external iliac artery and vein, and the AKT ureter was anastomosed with the recipient's bladder. Median AKT preservation time was 15 [14–17] hours.

Immunosuppressive therapy

In order to prevent graft rejection, 18 recipients (78%) received induction immunosuppressive therapy (IST) – monoclonal antibodies in 15 recipients (65%) and polyclonal antibodies in 3 (13%). Basic triple-medication immunosuppressive therapy, consisting of calcineurin inhibitors, inosine monophosphate dehydrogenase or proliferative signaling inhibitors, and corticosteroids, was administered to all patients. So, 14 recipients (61%) received cyclosporine as the primary IST medication, and 9 (39%) received tacrolimus. Twenty recipients

(87%) received mycophenolic acid as the second IST medication, while three (13%) received everolimus.

Initial kidney transplant function was considered primary if there was diuresis and no dialysis was required within the first 7 days following KT; it was considered delayed if RRT dialysis was needed within the first 7 days after the procedure.

The software program Statistica for Windows v.12.0, developed by StatSoft Inc. (USA), was used to conduct a statistical analysis of data obtained. Nominal data were described with median (Me) and 95% confidence interval. The Kaplan–Meier estimate was used for survival analysis. Survival confidence intervals were considered by Weibull's method. Survival curves were computed starting on the day of surgery.

RESULTS

Sixteen recipients (69.6%) had primary initial AKT function, while 7 (30.4%) had delayed AKT function. At the time of hospital discharge, creatinine levels ranged from 84 to 133 μmol/L in 7 recipients, 134 to 200 μmol/L in 9 recipients, above 200 μmol/L in 6 recipients, and above 600 μmol/L in 1 recipient. In 16 recipients, blood creatinine levels decreased to less than 200 μmol/L throughout a range of 1 to 25 days, with a median of 7 (3–15) days. The levels took two to five months to return to normal in three patients (13%); in another three patients, blood creatinine dropped to 350 μmol/l.

In-hospital recipient survival was 96% (n = 22). In one case (4%), the recipient died from severe sepsis and multiorgan failure on the background of spontaneous colon perforation. One-year and 3-year survival rates were 84.8% [95% CI 72–95] and 79% [95% CI 65–92], respectively (Fig. 1).

There were 3 deaths in the late postoperative period (three-year period). Renal graft was adequately functioning at the time of their death. The causes of death were oncologic complications (n = 1), intestinal obstruction (n = 1), and pneumonia (n = 1). It should be noted that patient deaths occurred within the first year after transplantation.

One-year and 3-year AKT survival rates were 84.8% [95% CI 72–95] and 73% [95% CI 59–87], respectively (Fig. 2).

Long-term survival of both recipients and grafts is currently difficult to assess, as most recipients underwent KT after 2019. To date, there are two observations of 7-year survival in recipients over 70 years of age with functioning AKT.

DISCUSSION

Dialysis-based RRT methods significantly reduce the quality of life of patients with stage 4–5 CKD who endure persistent mental and physical discomfort [12–14]. Even with advancements in RRT dialysis, death rates are still high, particularly among elderly patients. The quality of life of hemodialysis patients is closely linked to a higher risk of death [15]. These considerations unquestionably help to expand the options for transplant treatment for CKD.

Our initial KT outcomes in patients aged ≥70 years are optimistic. In-hospital, 1- and 3-year recipient survival rates were 96%, 84.8% and 79%, respectively; AKT survival rates were 96%, 84.8% and 73%, respectively. It should be noted that patients were carefully selected for the KTWL, and if concomitant comorbid pathology was detected, it was corrected provided that patients were highly committed to therapy and strictly followed

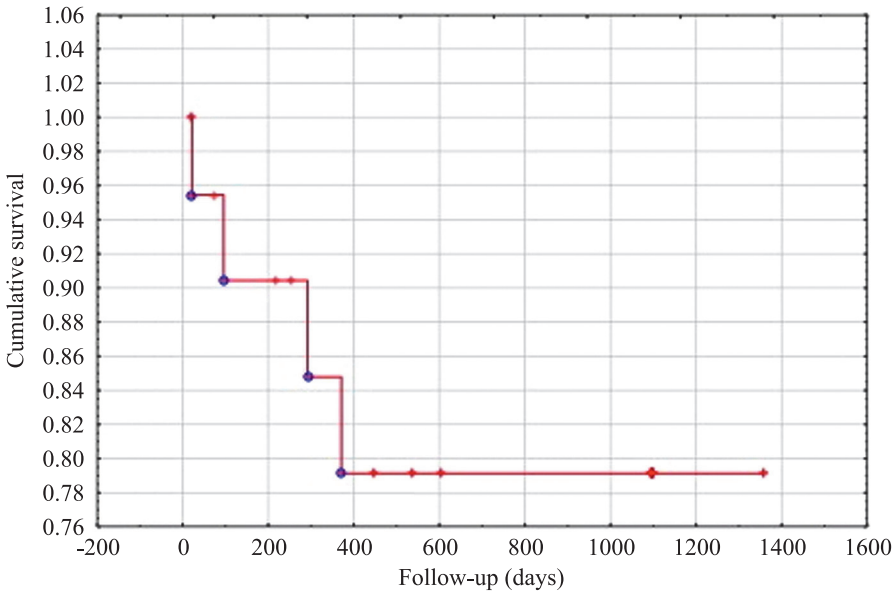

Fig. 1. Survival markers: completed follow-up (blue circle), censoring (red cutoff)

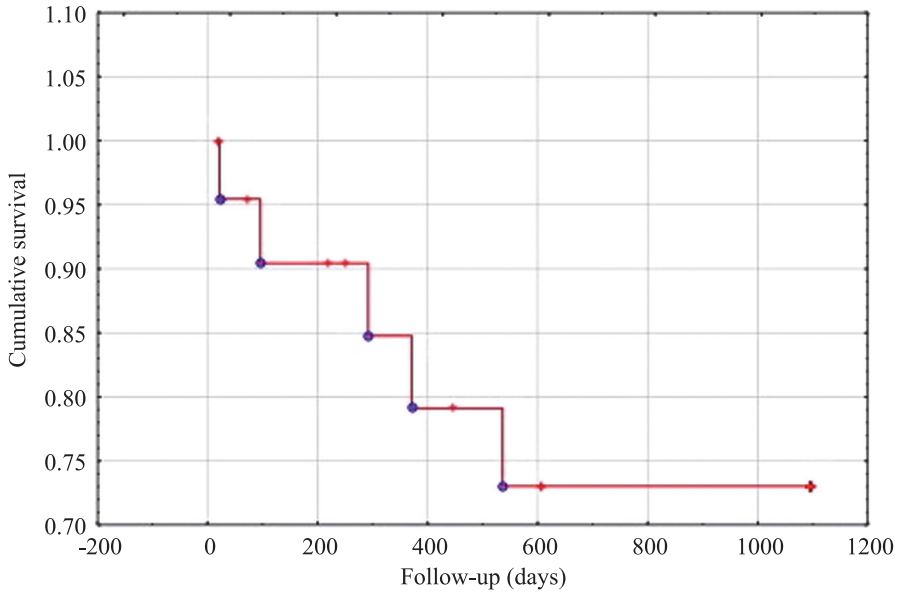

Fig. 2. Kidney graft survival. Survival markers: completed follow-up (blue circle), censoring (red cutoff)

guidelines. Given the significant risk of death in the first year following transplantation, a careless attitude to KT among patients aged 70 and older may lower their survival rate. For example, all deaths in the late postoperative period occurred within the first year after transplantation. A French study based on the REIN registry reported the importance of early referral of age-matched patients to a nephrologist, since nephrological 1-year follow-up before dialysis is linked to better survival and a higher likelihood of KT [16].

CONCLUSION

With a balanced approach and careful screening of potential AKT recipients for placement in the KTWL, KT is a viable and effective treatment modality for stage 4–5 CKD in individuals aged 70 years and above.

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FUNCTIONAL INDICATORS OF PERIPHERAL ARTERIAL STIFFNESS IN SOLID ORGAN RECIPIENTS (LITERATURE REVIEW)

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Increased arterial stiffness is an important preclinical indicator of cardiovascular dysfunction, arterial hypertension and target organ injury. This condition increases the risk of long-term adverse events. Solid organ recipients face multiple risk factors for cardiovascular complications due to transplant rejection, lifelong medication use and adaptive features of the transplanted organ. The review presents an analysis of the results of studies on the main functional indicators of peripheral arterial stiffness, as well as the potential effect of immunosuppressive therapy on indicators of vascular stiffness in solid organ recipients.

Keywords: stiffness, arterial stiffness, immunosuppression, recipients.

Increased peripheral arterial stiffness is a biological process associated with increasing age [1, 2], blood pressure [3], inflammation [4, 5] and vascular calcification [6]. Arterial stiffness may also be related to donor age [7], arterial stiffness index in a related kidney donor [8], post-transplant diabetes mellitus [9], cold ischemia time [10], graft function [11], hypomagnesemia [12], and graft rejection [13]. In addition, immunosuppressive therapy may affect arterial stiffness or rigidity [14].

Arterial stiffness or rigidity has become virtually synonymous with pulse wave velocity (PWV), the rate at which pressure waves move down the vessel. Theoretically, this can be demonstrated in such a way that in an elastic tube of homogeneous structure with cross-sectional area A filled with a fluid of density ρ, a perturbation in the system propagates as a wave along this tube at the PWV and is expressed by the Bramwell–Hill equation (Bramwell J.C., Hill A.V., 1922):

$$PWV = \sqrt{\frac{A\partial P}{\rho \partial A}},$$

where ∂A is the change in lumen area in response to a change in pressure ∂P.

$D = \frac{\partial A}{A\partial P}$, where D represents tube extensibility (defined as the relative change in cross-sectional area in response to pressure). Hence, $PWV = \sqrt{\frac{1}{\rho D}}$. Thus, higher vessel stiffness (lower D) results in higher PWV. Despite some limitations, PWV measurement has several advantages for clinical practice. When measured segment by segment (e.g., in the aorta), PWV gives an average value of its stiffness. In clinical practice, PWV is most often calculated using the formula PWV = ΔL/ΔT, where ΔL is the distance between two measurement points and ΔT is the time it takes for arterial pulse to travel from the proximal to the distal measurement point [15]. The aorta is the main elastic vessel in the body, PWV in the aorta or in its segments probably represents the most informative parameter. The most widely used method for measuring aortic PWV is carotid-femoral PWV, the transit time of which is estimated from Doppler signals measured on the carotid and femoral arteries, which are relatively close to the aorta [15].

There are several measurement devices available, including commercially available systems such as Complior [16], Sphygmocor [17], Pulsepen [18] and others, as well as specially designed data acquisition systems (which, for example, have been used in population-based studies by Framingham [19] and Asklepios [20]). Ideally, measurements are performed simultaneously; sequential measurements together with ECG synchronization are the best alternative method. Any ultrasound machine with a vascular probe can also be used, provided the methodology is followed and appropriate software is available. Difficulties arise when estimating the carotid-femoral pathway length: the intra-arterial distance must be estimated from measurements at the body surface, and the pulse wave does not travel strictly straight along a single pathway from the carotid artery to the femoral artery measurement site.

Although there are a number of different ways to determine the distance, which makes it challenging to standardize measurements, PWV is now often calculated

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by multiplying 0.8 by the distance between the common carotid artery and the common femoral artery measurement site [21]. Carotid-femoral PWV is considered the reference standard for clinical trials in Europe and the United States because of the availability of a large database of reference values obtained throughout Europe [22] and studies demonstrating the prognostic significance of this parameter [23–26].

PWV measurements should be evaluated according to patient age. The 2007 European Society of Cardiology Guidelines defined a fixed threshold value of 12 m/s to identify patients at high cardiovascular risk [27]. Later, expert consensus set this value at 10 m/s [21].

Another indicator reflecting arterial stiffness is the augmentation index. The augmentation index is based on pulse wave reflection and is a common measure of arterial stiffness. The augmentation index is defined as the ratio of the augmentation of systolic blood pressure to pulse pressure. To calculate it, it is necessary to determine the point of confluence of the direct and reflected waves (inflection point). According to some observations, this inflection point corresponds to the blood flow velocity peak and several algorithms have been developed for its identification [28–30].

Characteristic impedance is another arterial stiffness indicator. It relates absolute arterial pressure at a certain location to absolute blood flow velocity at the same location in the absence of reflected waves [31]. Characteristic impedance (Zc) is related to PWV by the formula: Zc = СПВ × ρ. Since blood density is approximately equal to unity, these values are numerically almost identical when expressed in cm/sec or in dyn·s per cm^3 [31]. It is virtually impossible to measure characteristic impedance by non-invasive methods because of the difficulty in excluding reflected wave effects and errors in non-invasive measurement of blood flow velocity and pressure.

OTHER ARTERIAL WALL ELASTIC PROPERTIES

1. Arterial distensibility – the change in relative diameter (or area) with increasing pressure. It is inversely related to the modulus of elasticity.
   $\Delta D/\Delta P \times D$ ($mmHg^{-1}$).
2. Distensibility coefficient – the relative change in vascular cross-sectional area per unit pressure
   $DC = [\Delta A/A]/\Delta P = 2 \times \Delta d/d/\Delta P$.
3. Compliance – the absolute change in diameter (or area) at a given pressure and a given vascular length
   $\Delta D/\Delta P$ (cm/mmHg) or (cm^2/mmHg).
4. Bulk modulus of elasticity – the pressure step required theoretically to increase volume by 100%
   $\Delta P/(\Delta V/V)$ (mm Hg) = $\Delta P/(\Delta D/D)$ (mmHg), without changing length.
5. Elastic modulus – the pressure step required theoretically to stretch the diameter 100% from a resting state at a fixed vascular length
   ($\Delta P \times D/\Delta D$) (mmHg).

PERIPHERAL ARTERIAL STIFFNESS AND IMMUNOSUPPRESSIVE THERAPY

It is known that pathological processes in the vascular wall are triggered by dysregulation of matrix metalloproteinase activity. The effect of immunosuppressive drugs on the activity of matrix metalloproteinases has been reported. Results from a study by Korean authors that examined the effects of cyclosporine on human umbilical vein endothelial cells revealed that the immunosuppressive agent activates most matrix metalloproteinases in endothelial cells, with the exception of MMP-2 [32]. A British study on laboratory animals examined the effect of cyclosporine, tacrolimus and rapamycin on intimal hyperplasia, expression of fibrosis-associated genes and deposition of extracellular matrix proteins. In all groups, there was a significant inhibition of matrix metalloproteinase (MMP)-2, MMP-9, tissue inhibitor of metalloproteinases (TIMP)-1, transforming growth factor (TGF)-beta and collagen III expression ($P < 0.001$) after 14 days, but deposition of extracellular matrix deposits increased [33]. Bianchi et al. investigated how cyclosporine affected the expression of vascular endothelial growth factor (VEGF) and MMP-2 in rat myocardium. In contrast to the control group, cyclosporine-treated laboratory mice showed structural myocardial changes, including fibrosis and degeneration as well as a significant increase in both MMP-2 and VEGF [34].

In a Dutch study including a cohort of 330 kidney transplant patients, PWV was found to be a prognostic factor for cardiovascular events, outcomes and survival, irrespective of patient age. Patients with a PWV of 7.5 m/s or higher had worse survival than patients with a PWV <7.5 m/s [35]. In a 2011 prospective study including 512 renal transplant recipients, PWV, central augmentation pressure, and augmentation index were measured at the time of renal transplantation. The mean follow-up period was 5 years, PWV and augmentation pressure were included in a model based on clinical variables and laboratory data to predict cardiovascular events. The addition of PWV and augmentation pressure data resulted in a 15.9% update and reclassification of cardiovascular events. Moreover, patients with a PWV of 8.1 m/s or higher had worse cardiovascular survival compared to patients with a PWV <8.1 m/s [36].

A study by Norwegian authors including 1,022 renal transplant recipients showed that below a threshold of 12 m/s, each 1 m/s increase in PWV starting at 8 m/s was associated with a 36% increase in mortality risk [37]. The presented study results demonstrate that PWV is a strong predictor of cardiovascular events and death, independent of age and other clinical or laboratory variables. The results also validate the findings of other studies that have been conducted involving different patient populations [38–40].

Results from early studies on the effects of calcineurin inhibitors on large artery function were conflicting. In a prospective study, Zoungas et al. compared PWV before and after renal transplantation in 36 patients [41]. Twelve months after transplantation, PWV improved in all patients regardless of the use of cyclosporine or tacrolimus, although the decrease in augmentation index was greater in patients receiving tacrolimus (8.0 ± 16.5% *vs.* 27.4 ± 18.2%; P = 0.01). In a small study by Covic et al., it was demonstrated that cyclosporine dramatically reduced the augmentation index [42]. However, the study lacked a control group, and the decrease in augmentation index after cyclosporine administration was linked to shorter reflected wave time, which may lead to increased PWV in the long term.

In the same period, a parallel study (including 250 stable renal transplant recipients) showed that cyclosporine increased augmentation index and blood pressure to a significantly greater extent than tacrolimus [43]. In 2007, Strozecki et al. compared PWV in 76 patients receiving cyclosporine and 76 patients taking tacrolimus [44]. The two study groups were matched for key clinical characteristics (age, blood pressure, duration of hemodialysis, diabetes mellitus). Higher PWV were found in the cyclosporine group compared to the tacrolimus group (9.33 ± 2.10 versus 8.54 ± 1.35, respectively; P < 0.01). In another study by the same authors using stepwise multiple regression analysis, it was observed that age, male sex, mean arterial pressure, cyclosporine (compared with tacrolimus), and fasting glucose levels were independently linked to higher PWV [45].

The effect of cyclosporine on arterial stiffness is probably due to increased vascular tone or impaired vasodilatory properties of nitric oxide. Given that cyclosporine administration is associated with higher PWV, switching to tacrolimus may reduce arterial stiffness. This hypothesis was tested in a small study where stable kidney recipients who had been taking cyclosporine for more than 10 years were converted to tacrolimus. PWV and ambulatory daily blood pressure monitoring (ABPM) were measured at baseline and 3 months after conversion, and no differences were found in either blood pressure or PWV, probably due to the short time interval after drug change [46]. All the studies cited suggest a possible negative effect of calcineurin inhibitors, especially cyclosporine, on PWV. In a randomized clinical trial, 17 of 27 patients were switched from cyclosporine to everolimus 6 months after kidney transplantation. PWV remained stable in the everolimus group (9.50 ± 1.92 *vs.* 9.13 ± 1.62 m/s, ΔPWV –0.37 ± 1.14 m/s), whereas it was elevated in the cyclosporine group (9.93 ± 1.94 *vs.* 10.8 ± 2.24 m/s, ΔPWV +0.89 ± 1.47 m/s) [47].

In a study by Gungor et al., no benefit in PWV or augmentation index (Aix) was found in patients treated with mTOR inhibitors (at least 6 months – either sirolimus or everolimus) compared with treatment with calcineurin inhibitors (cyclosporine or tacrolimus) [48]. In linear regression analysis, only classical risk factors (age, blood pressure, cholesterol level, and proteinuria) were found to be predictors of arterial stiffness. In a more recent randomized clinical trial, the effects of switching late from calcineurin inhibitors (tacrolimus) to mTOR inhibitors (everolimus) were examined. The findings demonstrated that left ventricular hypertrophy decreased in both groups. As secondary outcomes, changes in blood pressure (ABPM) and PWV were measured both before and after switching. The tacrolimus group (25 patients) and the everolimus group (31 patients) had median post-transplant durations of 1.7 and 1.3 years, respectively. Despite the fact that most patients receiving everolimus had dipper status, blood pressure in both groups was very well-controlled 24 months after randomization; 30% of those receiving tacrolimus were non-dippers, compared to 22% receiving everolimus. PWV at baseline, at 12 and at 24 months were within the normal range, with no significant differences between the two groups [49].

Another study evaluated PWV and blood pressure (ABPM) [50]. PWV was measured in 277, 223 and 184 patients after 12 and 24 months. Patients who switched to everolimus had a slight decrease in PWV (month 12: 0.24 m/s; month 24: 0.03 m/s), whereas patients taking cyclosporine showed a progressive increase in PWV (month 12: 0.11 m/s; month 24: 0.16 m/s); baseline values were within normal limits (mean 7.8 m/s for the everolimus group and 7.6 m/s for the cyclosporine group). Follow-up at 24 months confirmed the prognostic value of PWV, as the incidence of cardiovascular events in the entire cohort was low (2.8% in the everolimus group and 4.8% in the cyclosporine group). Since even small changes (0.4–0.5 m/s) usually occur over a long period of time, the follow-up period (at 24 months) was probably insufficient to detect any significant changes in PWV [51, 52]. Since patients with initially high PWV tend to have a more rapid increase in PWV [53], it is possible that switching to mTOR inhibitors may be beneficial in this patient cohort.

CONCLUSION

Stiffness or rigidity of the elastic and muscular-elastic arterial wall is a known independent predictor of adverse cardiovascular events [54–56]. Clinical practice has widely adopted noninvasive methods for examining systemic and local stiffness based on the measurement of PWV, augmentation index and other indicators [57, 58]. Published reports on mechanisms of regulation and the effect of immunosuppressive medications on vascular wall stiffness markers suggest that reducing arterial stiffness may be a therapeutic target for improving the quality of life in solid organ recipients [59–62].

The authors declare no conflict of interest.

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BK VIRUS NEPHROPATHY IN KIDNEY TRANSPLANTATION (LITERATURE REVIEW)

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The article presents a review of the literature on the current problem of modern transplantology – BK viral nephropathy after nephrotransplantation. Risk factors for BK virus reactivation in immunocompromised patients are reflected. The issues of screening and diagnosis of BK viral infection in people with a transplanted kidney are considered. The role of BK viral nephropathy in renal graft loss is emphasized. The clinical manifestations and treatment strategies of BK viral nephropathy in kidney transplantation are discussed.

Keywords: BKV, polyomavirus, kidney transplantation, BK viral nephropathy, diagnosis, treatment.

INTRODUCTION

BK virus (BKV, also known as polyomavirus) is a small non-enveloped virus with a circular, double-stranded DNA that belongs to the Polyomaviridae family. Its strains are classified into six genotypes according to the VP1 and NCCR polymorphisms. The four classified genotypes of BKV result in predominantly asymptomatic infections in childhood [1, 2]. Viral agnoprotein plays a key role in the BKV infectious cycle – in the assembly, morphogenesis and release of virions. About 80–90% of the population is seropositive for BKV. The main transmission routes are contact with mucous membranes, including the oral cavity, gastrointestinal tract, and respiratory tract [3]. After primary viremia, BKV remains in the kidney and uroepithelial cells, mainly in the parietal epithelium of Bowman's capsule, renal tubular epithelium and transitional epithelium, where it persists for a long time [4, 5]. BKV is capable of forming 40–45 nm intranuclear inclusion bodies in neuroepithelial cells of nephron tubules. Other localizations of latent BKV infection include the prostate, testicles, seminal tubules, cervix, vulva, and hematolymphoid tissues (peripheral blood mononuclear cells and tonsils) [6].

The virus is periodically reactivated and excreted with urine, but the infection remains asymptomatic in immunocompetent patients. Latent BK infection can become active when the functional activity of cellular immunity decreases against the background of immunosuppressive therapy or immunodeficiency states. BK infection has drawn increasing attention in recent decades due mostly to BKV-associated nephropathy (tubulointerstitial nephritis) resulting from profoundly compromised immune system [5, 7, 8]. BKV was first detected in 1971 in a renal transplant recipient with ureteral stricture. The first biopsy-confirmed case of BKV nephropathy was reported in 1993. It is debatable whether the rise in BKV incidence in subsequent years was the result of increased availability of reliable testing techniques for this infection or a consequence of the use of stronger immunosuppressive therapy regimens after kidney transplantation (KT). Because BKV-associated nephropathy (BKVN) frequently results in transplant rejection in patients, it is practically important to research its peculiarities. The final stage of kidney damage by BKV is characterized by interstitial fibrosis and tubular atrophy, accompanied by progressive nephron loss, impaired renal graft function and decreased graft survival [9].

In the first years after it has been reported, BKV nephropathy caused graft rejection in 50–100% of cases. However, as the role of BKV in posttransplant complications was recognized, the incidence of BKV-associated graft loss significantly decreased to 1–10%, although the 1-year incidence of graft loss ranges from 30% to 65% [10–12]. According to a recent study by Thorndyke et al (2023), the post-KT incidence of BKV nephropathy was 17.6%, with an 8.8% incidence of coinfection with cytomegalovirus [13]. Although BKV nephropathy is primarily seen in renal transplant recipients, cases have been reported in the kidneys of individuals with severe immunodeficiency [14, 15].

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The **objective** of the study is to examine current literature sources and summarize information about the nephrotoxic effects of immunosuppressive therapy.

MATERIALS AND METHODS

Scholarly articles in Russian and English dedicated to the issues of BKVN following KT were found using the search databases of Pubmed, Elsevier, Springer, and Elibrary. The search depth was 2017–2023. Keywords such as BKV, polyomavirus, kidney transplantation, BK viral nephropathy, diagnosis, and treatment were used in the search. The review included retrospective, prospective, analytical, descriptive studies, clinical guidelines, dissertation papers, systematic reviews and meta-analyses providing information on the principles of managing patients with BKV nephropathy in a transplanted kidney. Exclusion criteria for the review included conference abstracts, letters to journal editors, and papers published before 2017. A total of 77 publications were included in the present analysis.

RISK FACTORS FOR BK VIRUS REPLICATION IN KIDNEY TRANSPLANTATION

Because cellular immunity is most suppressed in the first year after transplantation as a result of induction therapy, it is during this period that the risk of BKV replication is increased [16], with 54% of cases occurring within the first 2–6 months after transplantation [13, 17].

A systematic review and meta-analysis of 34 publications presented by B. Demey et al. (2018) reported that tacrolimus regimen, deceased donor, male recipient, history of previous transplant, age at transplantation, ureteral stent use, delayed graft function, and acute rejection episodes increased the risk of BKV viremia to varying extents [18].

Similar data were obtained in a study by Alonso et al. (2022), who found that male sex (odds ratio [OR], 4.226; 95% confidence interval [CI] 1.660–10.758, p = 0.002), age (OR, 1.047; 95% CI, 1.008–1.088; p = 0.018) and retransplant (OR, 4.162; 95% CI, 1.018–17.015; p = 0.047) were independent predictors of BK polyomavirus infection [19].

Another study analyzing the clinical and laboratory data of 195 renal transplant recipients showed that deceased donor, decreased levels of direct bilirubin and blood neutrophils were risk factors for BK infection activation [20].

The intensity of immunosuppression is considered a key factor linked to BKV replication in kidney transplant recipients [16, 21, 22]. Immunosuppressive medications are known to have varying levels of immunosuppression. Research suggests that tacrolimus is associated with a higher risk of BKV reactivation in mammals than cyclosporine and mTOR inhibitors [18, 23, 24]. Against the background of such therapy, the virus can reactivate, induce lysis of tubular epithelial cells and release BKV virions into the bloodstream, causing various tubular and interstitial lesions with subsequent severe complications.

Recipient characteristics that increase the risk of BKV-induced nephropathy include advanced age, diabetes, and specific HLA-C alleles [18, 22]. At the same time, McCaffrey et al. (2021) has reported that BK infection is associated with younger patient age before transplantation, along with the recipient's negative serostatus for cytomegalovirus [21].

On the donor side, factors such as reduced immune response to BKV and BK-viruria prior to transplantation contribute to virus reactivation [25].

Donor-recipient interactions: high-risk serologic status in BKV-positive and BKV-negative donors, ABO incompatibility, HLA mismatch, reduced graft function, rejection or ischemia of the transplanted kidney, and ureteral stent increase the risk of BKVN in a transplanted kidney [26].

Another potential risk factor for BKVN is congenital anomalies of the kidneys and urinary tract. According to Avcı et al. (2022), among children aged 0–18 years who received renal transplants, the incidences of congenital kidney and urinary tract anomalies were 30.3% and 66.6% in those without and with BK polyomavirus infection, respectively ($p < 0.05$) [27]. The incidence of cytomegalovirus infection was significantly higher in the BK polyomavirus-positive group than in the non-infected group ($p < 0.05$).

The pathogenesis of BKV infection is presented in Figure.

CLINICAL MANIFESTATIONS OF BK POLYOMAVIRUS-ASSOCIATED NEPHROPATHY

Clinically significant BK infection occurs in renal transplant recipients due to reactivation of latent infection or transmission of new infection from the donor kidney [28, 29]. Stages of BK infection include viruria, viremia, and allograft nephropathy [26]. Persistent viruria in immunocompetent individuals can progress to viremia, which is initially asymptomatic [30]. Compared to viruria, viremia has been found to be a more accurate indicator of BKVN [31, 32].

The prevalence of viremia and BKVN is 10–15% and 3–5%, respectively [33]. According to other reports, viruria and viremia are detected in about 30% and 12% of renal transplant recipients, respectively [1, 31]. In a study of 326 transplants including 246 patients, Bicalho et al. (2018) found that the prevalence of viruria was 36.9%, viremia was 22.3%, and nephropathy was 3.2% [34]. Nearly half of kidney transplant recipients develop viremia within 2–6 weeks of the onset of viruria, and a comparable percentage of patients who have viremia also experience BKVN within the same time period [31, 35]. There are reports that viremia affects 10–30% of recipi-

ents in the first 6 months following transplantation and in 5–10% of recipients thereafter [32, 36].

BKVN usually occurs after a period of sustained, progressively increasing viremia, which is characterized by impaired kidney function with or without urinary dysfunction. Ureteral stenosis and hemorrhagic cystitis are other manifestations of BKV, although they are less common in renal transplant recipients [37].

Given its prolonged persistence in the genitourinary epithelium, there has been discussion on a potential link between BKV and genitourinary malignancies in renal transplant recipients [38]. Animal and *in vitro* studies demonstrate that BKV causes oncogenesis and cell transformation [39]. However, findings are unclear since BKV nephropathy patients have reduced cellular immunity, which itself is a risk factor for malignant neoplasms.

SCREENING AND DIAGNOSTIC OPTIONS FOR BK VIRUS

Screening kidney recipients for BKV infection and renal dysfunction at 1, 3, 6, 9, 12 months after KT allows to reduce immunosuppressive medication and promptly assess the risk of BKV injury to the graft [28, 33, 40]. According to international guidelines, screening should be done monthly for the first 6 months after transplantation and then every 3 months for the next 18 months [1, 22, 41]. It should be noted that such screening tactics are cost-effective. Compared with no screening, the incremental benefits of screening were 0.294 life-years saved and 0.232 quality-adjusted life-years saved. Total savings from screening were A$6986 (US$5057) [42].

Following a reduction in immunosuppressant dosage, renal function, medication levels, and viral load should be monitored. High levels of BK viremia have been linked to higher incidence of BKVN and increased incidence of acute rejection and overall worse graft survival (OR 1.988; 95% CI 1.012–3.907; p = 0.046) [19]. However, with this modification to therapy regimen, the elevated risk of kidney transplant rejection should be considered.

BKV viral load is measured by polymerase chain reaction (PCR). Differences in DNA extraction methods, sample type/source, primer and probe sequences, and variation in BKV genotype all influence assay results [43–45]. The results of assays conducted in different laboratories may differ as a result of these factors [46].

BKV nephropathy should be suspected when the plasma BKV load is ≥10,000 copies/mL. According to Bicalho et al. (2018), the cut-off value of viremia that best discriminates the progression to sustained viremia and to BK polyomavirus-associated nephropathy was 37,488 and 44,956 copies/mL, respectively [34]. Based on analysis of 393 time-matched urine and plasma samples collected after KT, Brochot et al. (2019) identified a viruria threshold of 6.71 $\log_{10}$ copies/mL as the best threshold for diagnosing BKVN (sensitivity 90.9% (95% CI 86.5–95); specificity 90.3% (95% CI 86.3–94.3) [47].

Cytologic analysis is the most specific and easy method of examining urine sediment. Typical BKV-infected

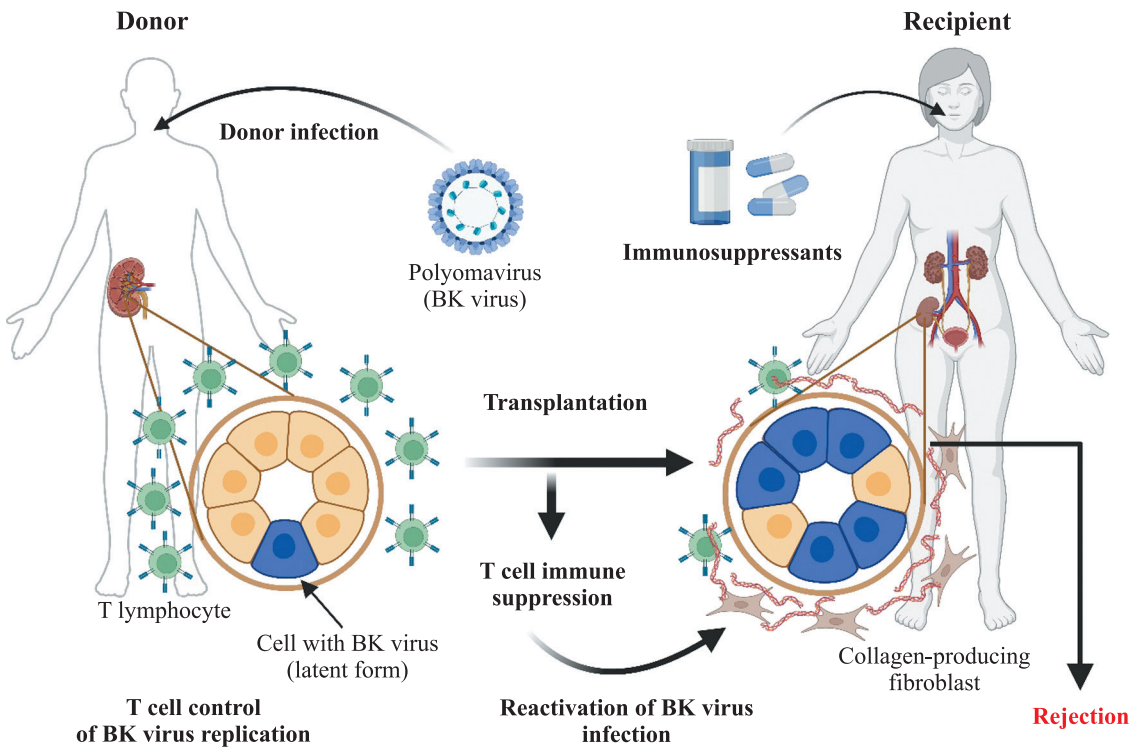

Fig. Pathogenesis of BK virus infection and its induced nephropathology in kidney recipients. The figure was prepared using online program BioRender (www.biorender.com)

cells found in urine cytology are called decoy cells because of their similarity to renal carcinoma cells, which can make differential diagnosis challenging [48]. They are tubular epithelial or urothelial cells with ground-glass-like nuclear inclusions surrounded by a condensed chromatin rim. They may also have "owl's eye" inclusions, multinucleation, or clumped chromatin. Although trap cells are a marker of BKV replication, they do not necessarily indicate BKV infection, as false-positive results are possible in transplant patients [49]. Nevertheless, the absence of trap cells in urine cytologic examination has a high negative predictive value for diagnosis of BK infection [50].

Non-invasive markers of BK infection are cylinder-like aggregates of mature virions and Tamm–Horsfall protein (uromodulin), which can be detected in urine samples, for example, by negative-staining electron microscopy [51]. Their presence or absence has an extremely high positive and negative prognostic value for diagnosis of BKV nephropathy, and the amount of BK Haufen shedding correlates significantly with severity of the disease, degree of lysis of tubular epithelial cells, as well as presence of trap cells, viruria, and viremia in the urine [52].

Kant et al. (2020, 2021, 2022) evaluated the association of donor-derived cell-free DNA (dd-cfDNA), BK viral load in recipient plasma with biopsy results [53–55]. It is known that dd-cfDNA circulates in the recipient's bloodstream and can be quantified by droplet digital PCR after targeted multiplex pre-amplification. Higher levels of dd-cfDNA were shown to correlate with higher BK viral load as well as histological changes diagnosed at biopsy that met Banff criteria for T-cell-mediated rejection. The authors concluded that dd-cfDNA levels may be an informative noninvasive test to assess BKV progression to BKVN.

Renal allograft biopsy with confirmation of interstitial nephritis and viral-induced cytopathic changes is currently the gold standard for diagnosing BKVN. This procedure not only enables the diagnosis of BKVN but evaluates the severity of the viral lesion and the existence of other concomitant lesions [1, 22]. Biopsy is conducted when there is persistent viremia – two or more viremia above 10,000 copies/mL [34].

However, it could be challenging to confirm the presence of BKV histologically. When sampling from unaffected renal parenchyma, the random and focal nature of the infection, especially in the early stages, may lead to false-negative results. Since BKV is tropic to the kidney medulla, it is necessary that the biopsy specimen contains the medulla to minimize the likelihood of sampling error [56, 57]. According to some reports, BKVN is undetected in nearly 30% of cases where biopsy samples are taken incorrectly. If the initial biopsy does not confirm the presence of BKVN, but there are clinical manifestations of the disease, a follow-up biopsy is advised.

The histologic picture of BKVN is similar to that of acute transplant rejection, which makes early identification more challenging. In both cases, the key histological manifestations are tubular injury, tubulitis, and interstitial inflammation. These are regarded as acute cellular rejection when there are no additional morphological or immunohistochemical signs of BK infection [58]. Endarteritis, arterial fibrinoid necrosis, glomerulitis, or C4d staining of peritubular capillaries are among the signs of vascular injury that are typically more consistent with acute rejection than with polyomavirus infection [58]. According to Yang et al. (2022), high-frequency ultrasound can be used to differentiate between BK polyomavirus-associated nephropathy and renal graft rejection: the presence of eccentric hydronephrosis and subcapsular hypoechoic areas has a high specificity [59]. Undoubtedly, histologic data should be correlated with the history of the disease and the results of additional laboratory tests, primarily BKV load and the presence of donor-specific antibodies.

Auxiliary tests, such as immunohistochemical staining or *in situ* hybridization can be used to improve the accuracy of BKV diagnosis in biopsy specimens [57, 60]. Immunohistochemical staining enables the detection of BKV at early stages of infection, even before the development of characteristic cytopathic changes on conventional staining, and also allows differentiating BKV from other viral nephropathies observed in immunocompetent patients (adenovirus, cytomegalovirus infection, etc.). Detection of the large SV40 T-antigen (homologous to BKV polyomavirus Simian Virus 40) indicates active BKV replication, the amount of which reflects viral load. When evaluating staining with SV40 T antibodies, the reaction intensity is expressed as a score (0–3), and the percentage of tubules with cell staining (<1%, ≥1% and ≤10%, >10%) and the percentage of stained tubular cells are also taken into account [57].

Several grading systems have been proposed for assessing BKV severity; the Banff Working Group system is one of the most popular [57, 61]. The Banff Working Group Histological Classification for polyomavirus nephropathy is a three-level approach that takes into account the degree of morphological features of BK infection, the intensity of interstitial fibrosis, and the degree of viral load (Table).

Renal graft function is most impaired in class III patients and their prognosis is significantly worse.

Thus, characteristic cytopathic changes and positive immunohistochemical tests using antibodies specifically directed against BKV or against the cross-reactive large SV40 T antigen must be present for a conclusive diagnosis of BKV-induced nephropathy in a transplanted kidney [62]. Research on potential biomarkers of BKVN in a kidney transplant appears to be relevant and valuable.

Table

Banff Working Group Histological Classification for polyomavirus nephropathy [61]

Classes of polyomavirus nephropathy					
Class I		Class II		Class III	
pvl	Banff ci score	pvl	Banff ci score	pvl	Banff ci score
1	0–1	1	2–3	–	–
–	–	2	0–3	–	–
–	–	3	0–1	3	2–3

Note: pvl denotes the polyomavirus replication/load level, calculated as follows: pvl1: ≤1% of all tubules/ducts with polyomavirus replication; pvl2: from >1 to ≤10% of all tubules/ducts with polyomavirus replication; pvl3: >10% of all tubules/ducts with polyomavirus replication; Ci denotes interstitial fibrosis: Ci0: interstitial fibrosis in ≤5% of the cortex; Ci1 denotes interstitial fibrosis in >5% and ≤25% of the cortex; Ci2 denotes interstitial fibrosis in >25% and ≤50% of the cortex; Ci3 denotes interstitial fibrosis in >50% of the cortex.

APPROACHES TO THE TREATMENT OF BKVN, INCLUDING IN TRANSPLANTED KIDNEY

Reducing the immunosuppression intensity while keeping an eye on the viral load in urine and/or blood is a fundamental principle in the treatment of BK-viremia and BKVN, although it is associated with the risk of acute rejection after treatment [22, 63, 64]. To lessen immunosuppression, the following strategy has been suggested [16]:

1. Cutting the immunosuppressive dosage by half against the background of previous doses of calcineurin inhibitor and/or prednisolone, while checking serum creatinine and viral load levels by plasma PCR in the same laboratory every 2 weeks.
2. If BK viral load stays the same or rises, the immunosuppressants should be discontinued completely.
3. If the viral load does not decrease within 4 weeks despite discontinuing the immunosuppressant (4–6 ng/mL for tacrolimus and 50–100 ng/L for cyclosporine), a reduction in calcineurin inhibitor target values is recommended.

Quinolones, cidofovir, leflunomide, and intravenous immunoglobulin are additional therapies for BKV infection [16]. It should be noted that among these medications, only intravenous immunoglobulin has an evidence base for efficacy against BKV infection [65–67].

Intravenous immunoglobulin is given where maximum reduction of immunosuppression fails [65]. This treatment strategy is justified by the fact that intravenous immunoglobulin preparations contain BKV-neutralizing antibodies [68]. In a pediatric population of kidney recipients on the background of intravenous immunoglobulin treatment, Mohammad et al. (2022) reported that viral resolution was achieved in 70% and that no difference was noted in estimated glomerular filtration rate between BKV and non-BKV group (p = 0.438). There were no rejection episodes and graft survival was 100% over median follow-up of 3 years [69].

Although quinolones (ciprofloxacin and levofloxacin) have been shown to have antiviral qualities *in vitro*, there is no convincing evidence to support their efficacy in preventing and treating BK virus infection following transplantation [70].

Although cidofovir, a cytosine nucleotide analog, has shown action against polyomaviruses *in vitro* [71], subsequent studies have demonstrated no benefit from cidofovir use. Moreover, cidofovir has been linked to proteinuria, proximal tubular dysfunction, and impaired renal function [72].

Teriflunomide (A771726), an active metabolite of the prodrug leflunomide, exhibits antiviral and immunosuppressive qualities. Despite initial enthusiasm for its use in BKV infection [73], the efficacy of leflunomide in BKVN is still debatable [74].

In the absence of developed and implemented antiviral agents with activity against BKV, the potential use of individually selected phytotherapeutic agents with antiviral properties should be considered. For example, San-Yuan Chen et al. (2017) found that extracts of *Rhodiolae Kirliowii Radix et Rhizoma* and *Crataegus pinnatifida* fruits inhibited BKV cell infection, as evidenced by reduced expression of viral proteins VP1 in BKV-infected renal epithelial HK-2 cells. The calculated 50% effective doses against BKV were 21.68 μg/mL for *Rhodiolae Kirliowii* extract and 65.54 μg/mL for *Crataegus pinnatifida* extract. The cytotoxicity study showed that at concentrations of 300 μg/mL, the studied extracts did not harm kidney cells [75].

Patients with BKVN-associated graft loss should be considered for re-transplantation, given the strong evidence supporting its effectiveness, [76, 77]. One-year allograft survival in BKVN patients who undergo re-transplantation is 91% [76].

CONCLUSION

BKV infection continues to be one of the most common clinical challenges in transplantology. There are numerous risk factors for BKV reactivation. Posttransplant monitoring of BKV reactivation, which should include searching for “trap cells” in urine and assessing viremia by PCR is the cornerstone of BKVN prophylaxis. BKVN treatment is an unsolved problem since the key aspect is to reduce immunosuppression, which may lead to graft rejection. Antiviral medications designed to destroy BKV have not yet been used in clinical settings.

The authors declare no conflict of interest.

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PREOPERATIVE EXTRACORPOREAL MECHANICAL CIRCULATORY SUPPORT FOR PATIENTS WITH ACUTE SEVERE MITRAL VALVE REGURGITATION DUE TO PAPILLARY MUSCLE NECROSIS

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Background. Acute mitral valve insufficiency has a high mortality rate (up to 100%). Mechanical circulatory support and emergency surgery can improve the survival of this patient cohort. **Objectives:** to analyze a 12-year single-center experience of treating acute post-infarction mitral valve insufficiency. **Materials and methods.** This retrospective study included 12 adult patients with ST elevated myocardial infarction (STEMI) and corresponding acute mitral valve insufficiency who underwent surgery between 2009 and 2017. We analyzed the in-hospital period of all patients and long-term follow-ups whenever possible. All patients underwent preoperative coronary angiography and echocardiography. All patients underwent cardiopulmonary bypass and cold-blood cardioplegia. If venoarterial extracorporeal membrane oxygenation (VA-ECMO) was required, the femoral approach was preferred. **Results.** Seven patients needed VA-ECMO support, six of them preoperatively; four received mechanical circulatory support outside the hospital. All patients underwent percutaneous coronary intervention (PCI) with successful revascularization of the culprit artery. All but one patient underwent surgery within the first 24 hours. One patient underwent repeat surgery once the mitral valve could be repaired, and the other patient did not require any coronary bypass. In-hospital mortality occurred in one patient in the VA-ECMO group. Patients receiving VA-ECMO had longer duration of inotropic support, ventilation time, and intensive care unit stay ($p < 0.01$). **Conclusions.** Acute mitral valve insufficiency due to STEMI remains a dramatic complication, but the perioperative use of VA-ECMO helps reduce 30-day mortality and improve outcomes in this group of patients.

Keywords: acute mitral regurgitation, ECMO, STEMI.

INTRODUCTION

Acute progression of coronary artery disease typically presents with ST-elevation myocardial infarction (STEMI), and depending on the localization of ischemia, may lead to papillary muscle necrosis with subsequent rupture, which is often accompanied by leaflet prolapse and mitral valve regurgitation (MR) [1, 2]. Chronic ischemic mitral valve disease is the second largest cause of MR, accounting for 20% of all cases, preceded only by degenerative mitral disease (60–70%) [3, 4]. However, acute ischemic MR is relatively rare, and patients often present with cardiogenic shock and have high mortality [5]. For instance, Kettner et al. reported that the preoperative mortality in these patients was as high as 88%, with a 30-day mortality of 100% if no mechanical circulatory support was established [6]. Primary diagnosis of the acute state involves echocardiographic evaluation of the mitral valve, left atrium, and left ventricle. Furthermore, hemodynamic stabilization of the patient is the primary goal to prevent pulmonary congestion and development of pulmonary edema [7, 8]. To avoid further clinical deterioration, early implementation of venoarterial extracorporeal membrane oxygenation (VA-ECMO) in patients with acute myocardial infarction complicated by cardiogenic shock has been proven beneficial [9]. After hemodynamic stabilization, surgical treatment of the mitral valve has shown similar results in patients who undergo mitral valve repair and replacement [10]. Furthermore, simultaneous mitral valve repair and myocardial revascularization are not associated with better outcomes than revascularization alone after two years of follow-up [11]. As stated previously, VA-ECMO is the preferred therapy for patients with cardiogenic shock. However, literature on preoperative management of patients with severe acute ischemic MR is relatively scarce [12, 13]. In this study, we present and evaluate our experience with patients presenting with severe acute MR due to myocardial ischemia.

MATERIALS AND METHODS

This retrospective study was performed at the cardiac surgery department of our institute (Hannover Medical School, Hannover, Germany). The ethics committee of

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our institute waived the need for patient consent for this study.

Patient population

All patients who presented to our center between July 2009 and February 2017 with acute severe MR due to myocardial ischemia and those aged >18 years at the time of presentation were included in this study (n = 12). All data were retrieved through a retrospective review of patient records. Hospital databases, patient charts, surgical reports, and imaging data were reviewed. Patient follow-up was performed by telephoning the patient and referring them to the cardiologist and/or general practitioner. Postoperative diagnostic examination results were obtained via mail or fax.

Diagnosis and assessment of MR severity

STEMI was confirmed using electrocardiography (ECG). Ongoing myocardial ischemia was confirmed by clinical chemistry. Elevated values of troponin T and MB fraction of creatinine kinase were considered upon diagnosis. Coronary angiography was performed at our center or the hospital of primary presentation. Transesophageal echocardiography was performed prior to surgery to determine the grade of mitral valve regurgitation, cardiac orifice dimensions, and left ventricular ejection fraction (LVEF). The LVEF was determined using the modified Simpson's method, and MR was graded from 1+ to 3+ (mild, moderate, or severe) according to the American Society of Echocardiography guidelines [14]. The patient characteristics are presented in Table 1.

ECMO implantation

In all patients, the development of cardiogenic shock was rapid; therefore, end-organ perfusion assessment was not performed because of emerging circulatory failure. The decision to use VA-ECMO was mainly based on the patient's clinical evaluation results. The signs of cardiogenic shock according to SHOCK and IABP-SHOCK II trials, high doses of inotropes and vasopressors (epinephrine >0.3 mcg/kg/min in a combination with norepinephrine >1 mcg/kg/min) and rapid worsening of the hemodynamics despite pharmacological support were the indications for ECMO implantation.

All patients with preoperatively implanted ECMO at the end of surgery were switched back from cardiopulmonary bypass to VA-ECMO, per protocol. In other patients who had no mechanical circulatory support preoperatively, the decision to use VA-ECMO was made according to the hemodynamic situation at the end of the cardiopulmonary bypass, similar to the preoperative situation.

Cannulation was performed by a cardiac surgeon at the bedside in our department. After percutaneous placement of the guidewires in the common femoral artery (for both body and distal limb perfusion) and femoral vein, a single bolus of 5000 IU of unfractionated heparin was administered intravenously. The outflow cannula was implanted using Seldinger's technique into the femoral vein using a 55 cm long BIOLINE-coated HLS cannula (Maquet, Rastatt, Germany) with a size of 21, 23, or 25 F. Correct positioning of the outflow cannula just below the entrance of the inferior vena cava in the right atrium was proven using ultrasound. For the inflow cannula, a 15 cm long BIOLINE-coated HLS cannula (Maquet, Rastatt, Germany) with a size of 13, 15, or 17 F according to the patient size, was placed in the common femoral artery. A 7 F introducer sheath (Medicovation GmbH, Gladbeck, Germany) was used for distal limb perfusion. After successful cannulation, the cannulas were connected to our mobile ECMO system, CardioHelp pump with HLS Set Advanced 7.0 (Maquet, Rastatt, Germany) for those patients who required transportation from an external hospital, and to the PLS System with PLS Set Plus (Maquet, Rastatt, Germany) for others.

Surgical techniques

All patients were operated under combined general anesthesia via a median sternotomy using cardiopulmonary bypass and cold blood cardioplegia. In patients with preoperative ECMO implantation, a femoral venous cannula was also used for cardiopulmonary bypass

Table 1

Preoperative data of patients with acute mitral valve regurgitation due to STEMI

	Without ECMO	With ECMO	p
Age, yrs	60 (45.9–60.1)	60.1 (53–65.7)	0.17
EuroSCORE II, %	22.79 (21.79–30.11)	30.63 (25.19–33.12)	**0.04**
PCI preoperatively	100%	100%	–
EF, %	60 (50–60)	55 (40–60)	0.4
CK-MB, U/l	21 (13–32)	63 (41–586)	**0.03**
AST, U/l	30 (22–31)	1850 (112–8071)	**0.03**
LDH, U/l	392 (289–496)	2580 (717–9309)	**0.05**
CRP, mg/l	27.5 (8–123)	103 (67–369)	0.11

with additional cannulation of the superior vena cava; in others, a standard bicaval cannulation was applied.

In all but one patient, only venous grafts were used for myocardial revascularization because of an emergency. Distal anastomoses were performed before valve repair or implantation. Mitral valve access was achieved via the Soondergaard interatrial groove in all cases. Traditionally, mitral valve replacement has been performed with preservation of the posterior leaflet using 12–15 pledgeted sutures. We used biological or mechanical valves according to the actual guidelines [15].

Statistical analysis

Summary statistics were presented as medians and ranges. Categorical variables were presented as counts and percentages. Group comparisons were performed using Student's t-test for continuous variables. For categorical analysis, the Wilcoxon–Mann–Whitney test for small sample sizes was used. Statistical significance was set at $p < 0.05$ all tests. SPSS version 26 (SPSS Inc., Chicago, IL, USA) was used to analyze the data.

RESULTS

The mean follow-up period for the entire patient cohort was 1166 (998–2037) days. Table 3 shows all patients individually, including postoperative and follow-up details.

Most patients had inferior or posterior infarction (75%) and all developed symptoms of MR within one week after STEMI. Six of seven patients from the VA-ECMO group received circulatory support preoperatively; at the time of evaluation by the cardiac surgeon, two had Shock Stage E, and four had Shock Stage D. The other patients who did not receive ECMO preoperatively had Shock Stage C (SCAI shock staging) [16]. Four patients were assessed in a regional hospital by our team, received mechanical support there, and were transported using running VA-ECMO to our institute.

Table 2

Intraoperative data of operated patients

	Without ECMO	With ECMO	p
Mitral surgery	3 biological, 1 mechanical, 1 reconstruction	4 biological 3 mechanical	
CPB, min (median)	125 (113–143.5)	132 (117–146)	0.46
X-Clamp, min (median)	81 (65.75–70.5)	67.5 (62–70.5)	0.3
Grafts, (mean)	1 (0–2)	1.875 (0–3)	
Re-Operation	–	1 (14%)	
ECMO preoperatively	–	6 (86%)	
IABP	1 (14%)	–	

All but one patient underwent surgery within the first 24 hours after admission (93%) as a clear improvement of end-organ perfusion (i.e., increase in urine output and decrease in serum lactate) and a drop in pharmacological support. In one patient on VA-ECMO, redo surgery was performed 12 years after the previous myocardial revascularization. Intraoperative data are presented in Table 2.

As shown in the table, patients on VA-ECMO tended to have lower bypass and cross-clamp times, although this was not statistically significant. In one patient, the mitral valve could be reconstructed, and all other patients received either biological (five patients) or mechanical (six patients) valves. Two patients did not require coronary artery grafting because of previous complete endovascular revascularization.

However, the in-hospital mortality rate is low. Only one patient (8.3%) died on postoperative day 13 due to several severe ischemic complications: on VA-ECMO, she developed limb ischemia and had to be operated on to relieve the compartment syndrome; however, after VA-ECMO removal on day 5, she developed mesenteric ischemia, which caused her death.

The mean duration of mechanical circulatory support was 5 (3–9) days. Inotropic support was needed for 2.5 (1–4) and 8 (4–13) days after surgery in the non-ECMO and VA-ECMO groups, respectively ($p < 0.01$). Similarly, patients in the ECMO group required a longer mechanical ventilation time: 5 (1–8) versus 8 (4–13) days. New dialysis was required in four patients, all in the VA-ECMO group. One patient developed brain damage in the form of multiple small lesions. Eight patients (6 from the ECMO group) were transferred for weaning to other centers, either intubated or after dilative tracheostomy. Figure shows the differences in the biochemical markers between patients with and without mechanical support. Patients on VA-ECMO preoperatively had significantly higher levels of cardiospecific enzymes and markers of hepatic congestion, showing more severe disturbances in central hemodynamics and clarifying the implications of VA-ECMO. However, these patients do not show a secondary lactate peak representing reperfusion of the ischemic tissue after the operation because of sufficient preoperative circulatory support.

As seen in Table 3, only three patients, two without ECMO and one with VA-ECMO, were directly discharged. Others required longer weaning from ventilation and were transferred to other centers after tracheostomy. During the late follow-up period, six patients were lost due to multiple causes (e.g., foreign patients). One patient with known severe atherosclerosis required limb amputation at the knee level shortly after the initial surgery and another died several years later (detailed information was not available). Others were reported to be alive with

low NYHA grades and acceptable life quality, according to brief telephone communication.

DISCUSSION

The incidence of acute mechanical complications (MR, wall rupture) due to myocardial infarction is extremely rare and remains at 0.27% for STEMI and 0.06% for NSTEMI. Interestingly, there have been no significant changes in the incidence during the last 20 years, according to Elbadawi et al. [17]. In one of the latest published case reports, a 69-year-old woman with papillary muscle rupture after STEMI presented to the hospital with signs of cardiogenic shock; however, the only mechanical support she received was an intraaortic ballon pump [18]. Transfer to cardiac surgery or ECMO implantation were not initiated, leading to death. To demonstrate the maximal mechanical support and correct timing of surgery, we report our case series.

In our study, nine of the 12 patients had posterior myocardial infarction leading to rupture of the posteromedial papillary muscle, and all of them were percutaneously revascularized several days before developing heart failure. Six of the 12 patients required preoperative mechanical circulatory transport to survive until they could undergo cardiac surgery and one another received VA-ECMO at the time of admission to our center. The patients were divided into two clearly incomparable groups was done to emphasize the obligatory differences

Table 3

Follow-up summary

Pt	Age	Sex	MI site	Surgery	ECMO	Postoperative course	Follow up
1	60	M	Lateral	Mechanical MVR, CABG to 1, Closure of LAA	No	Extubated on POD 2, inotropic support till POD 4. Discharged on POD 8	Died late in follow up
2	45	M	Posterior	Mechanical MVR, CABG to LAD, OM-1 and RPD	Yes, 1 day before and 5 days after surgery	Extubated on POD 4, inotropic support till POD 6. Postoperative implantation of ICD. Discharged on POD 27	Lost
3	64	M	Posterior	Mechanical MVR, CABG to OM-1 and RCA, Closure of LAA.	No	IABP till POD 3. Extubated on POD 3, inotropic support till POD 4. Discharged on POD 21	Alive, uneventful
4	69	M	Lateral	Redo after CABG 12 years ago, Biological MVR	Yes, 2 days before and 4 days after surgery	Inotropic support till POD 7, new dialysis postoperatively. Transferred on dialysis and intubated for weaning on POD 8	Alive, right limb amputation due to atherosclerosis 3 months after surgery
5	48	M	Posterior	Biological MVR, CABG to PLA and RPLD	Yes, 1 day before and 4 days after surgery	Tracheostomy on POD 4. Inotropic support till POD 13. Dialysis till POD 20. Transferred intubated for weaning on POD 28	Lost
6	44	M	Posterior	Mechanical MVR, closure of LAA and PFO	No	Tracheostomy on POD 7, inotropic support till POD 3. Transferred for weaning on POD 15	Lost
7	52	M	Posterior	Biological MVR, CABG to LAD, OM, RPD	Yes, 1 day before and 5 days after surgery	Tracheostomy on POD 9. Limb ischemia with surgery. Transferred for weaning with mild inotropic support on POD 11	Alive, uneventful
8	66	F	Posterior	Biological MVR, CABG to 2	Yes, 5 days after surgery	Tracheostomy on POD 7. Transferred for weaning with mild inotropic support on POD 8	Lost
9	65	F	Posterior	Mechanical MVR, CABG to 1	Yes, 1 day before and 9 days after surgery	Limb and mesenterial ischemia, new dialysis postoperatively. Tracheal injury during emergency intubation	Died on POD 13 due to multiple ischemic events
10	60	M	Lateral	Biological MVR, CABG to 3	Yes, 1 day before and 3 days after surgery	Tracheostomy on POD 6. Multiple small ischemic lesions in the brain. Transferred for weaning on POD 8	Lost
11	62	F	Posterior	MV-Repair, CABG to 1	No	Extubated on POD 1. Inotropic support till POD 1. Discharged on POD 8	Alive, has developed lung cancer
12	53	M	Posterior	Mechanical MVR, CABG to 1	Yes, 1 day before and 5 days after surgery	Extubated on POD 4, inotropic support till POD 6	Lost

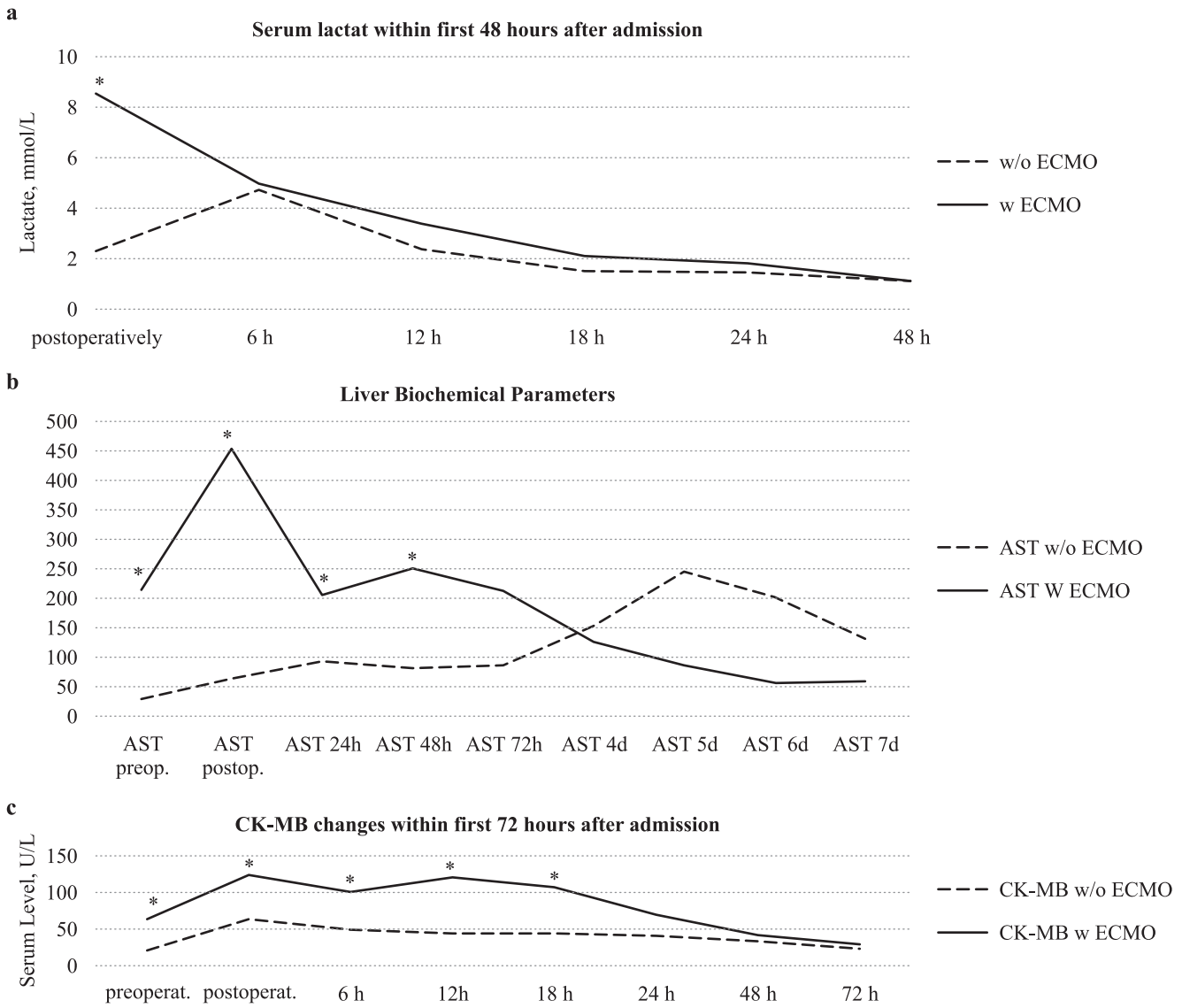

Fig. Changes in selected biochemical markers after the surgery: a – serum lactate; b – liver markers; c – CK-MB. The levels of serum lactate and liver markers suggest that patient with ECMO had more severe circulatory disturbance preoperatively, while higher CK-MB levels in the ECMO group could be explained by greater infarction area. * – $p < 0.05$

in perioperative management and follow-up depending on preoperative hemodynamic deterioration.

The clinical evaluation of patients showed that the EUROSCORE II suggested very high mortality rates in both groups, with a clear prevalence in patients needing mechanical support – 21.79% (19.89–26.86) versus 32.68% (21.79–52.43). The latter also showed significantly higher levels of hepatic and myocardial damage markers, which were recognized as indications for VA-ECMO implantation, along with a high need for inotropic support and clinical signs of cardiogenic shock. Mechanical circulatory support was used in all cases of SCAI Schock Stage D and higher to prevent prolonged end-organ hypoperfusion and avoid possible complications of high-dose combined inotropes and vasopressors. There is a possibility of early implications of mechanical circulatory support.

The postoperative course of lactate, myocardial, and liver markers showed that preoperative mechanical support allowed the restoration of an acceptable level of end-organ perfusion and discontinuation of the development of cardiogenic shock within several hours. In these cases, despite higher possibilities determined using EUROSCORE II (32.68%) and previously reported (up to 100%) [6, 13] mortality, 91.7% (11 of 12 patients) showed early overall survival. At the same time, the VA-ECMO implantation rate amounted to 58.3% (7 of 12 cases), which is significantly higher, than previously reported. We suggest that the early establishment of mechanical circulatory support in terms of VA-ECMO and time to hemodynamic stabilization is crucial in such patients.

CONCLUSION

Our preoperative ECMO implantation strategy could significantly improve the results in the high-risk group of patients with acute severe mitral valve regurgitation due to papillary muscle necrosis. The postoperative mortality in the ECMO group (8.3%) was significantly reduced in comparison to previous studies, based on the use of EUROSCORE II.

The authors declare no conflict of interest.

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PREDICTIVE VALUE OF NON-INVASIVE COMMON CAROTID ARTERY ELASTICITY IN HEART RECIPIENTS

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Objective: to study the predictive value of the local noninvasive elasticity index of the common carotid artery (CCA) wall in heart transplant recipients**. Materials and methods.** The study included 101 heart recipients. All study subjects underwent ultrasound examination of carotid arteries with assessment of the damping function of arteries and measurement of local and regional indicators of elastic properties of the CCA. The vascular wall elasticity index (iCOMPL) of the CCA was calculated according to the formula using the CCA diameters in systole and diastole and measurements of systolic and diastolic blood pressure. All-cause death, heart retransplantation, and clinically significant heart transplant coronary artery disease were evaluated as combined endpoints (adverse outcomes). **Results.** In heart recipients without morphological and immunohistochemical signs of heart graft rejection, detection of relatively low iCOMPL of CCA is a predictor of earlier development of adverse events ($p = 0.03$). Conclusion. iCOMPL of the CCA is a noninvasive and easily reproducible predictor of adverse outcomes in the long-term period after heart transplantation and can be used in clinical practice for the purpose of risk stratification in heart recipients.

Keywords: vascular wall elasticity, common carotid artery, heart transplantation, recipients.

INTRODUCTION

The issue of improving long-term prognosis and achieving active longevity in heart recipients are currently coming to the forefront and will actually dictate how the discipline of transplant cardiology develops going forward. Heart transplantation (HT) is still the gold standard for treating end-stage heart failure; the number of HT centers in the Russian Federation has increased, the number of HT operations has risen, medical care technologies have improved, and perioperative and in-hospital mortality has decreased tenfold, and rehabilitation programs have been developing. All these factors have led to a multiple increase in the number of heart recipients [1, 2]. At the same time, the success attained is largely attributable to the reduced rate of postoperative adverse outcomes, and further improvement in the long-term prognosis of heart recipients necessitates the development of strategies for risk stratification, prevention and prompt treatment of pathologic conditions in the long-term period.

The primary adverse factors affecting the long-term prognosis, aside from the irreversible risk of rejection, are cardiac graft cardiopathy and extracardiac complications brought on by immunosuppressive therapy side effects, as well as those linked to compromised cardiac reflexes, heart autonomic denervation, and chronic subclinical activation of the inflammatory system, which impairs vascular endothelium function [3].

According to our hypothesis, the indicators characterizing the elasticity of the arterial wall of large arteries can be used as noninvasive predictors of adverse outcomes and markers of various complications. The simplicity, reproducibility and low cost of the study of morphological properties of large arteries and physiological assessment of blood flow parameters using ultrasound suggests a high potential for their routine use [4–7].

In a previous single-center prospective observational study, it was shown that graft rejection in heart recipients is linked to changes in the elastic properties of the common carotid artery (CCA); the CCA arterial stiffness is increased in all types of acute graft rejection, and then decreases against the therapy [8, 9]. It was also found that the evaluation of the elasticity index of the common carotid artery, another ultrasonographic metric, can be used to assess the extent to which different factors negatively impact the main arteries in recipients of solid organs [10].

The **objective** of this study was to investigate the prognostic value of the local noninvasive elasticity index of the CCA in HT recipients.

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MATERIALS AND METHODS

The results of this study are based on analysis of the data obtained during case follow-up at Shumakov National Medical Research Center of Transplantology and Artificial Organs of 101 HT recipients who underwent orthotopic heart transplantation from March 2000 to April 2015.

All patients after transplantation received combined immunosuppressive therapy including tacrolimus, mycophenolic acid and glucocorticoids, as well as necessary adjuvant therapy as indicated.

Arterial wall elasticity indices were measured during routine examination after HT surgery between February 2013 and May 2015.

The exclusion criterion was the presence of signs of cardiac graft rejection detected by endomyocardial biopsy or acute infectious diseases.

The structural and functional characteristics of the CCA wall were determined using the Vivid S70N ultrasound diagnostic system with a 9 MHz linear multifrequency sensor measuring the thickness of the intima-media complex, determining the carotid-femoral pulse wave velocity (PWV), and calculating the elasticity index, hereinafter referred to as iCOMPL (**i**ndex **COMPL**iance). The vascular wall elasticity index was calculated using the formula.

$$iCOMPL = [(D_{dia}^2 - D_{sys}^2)/D_{dia}^2]/[(P_{sys} - P_{dia})/P_{sys}],$$

where D_{sys} and D_{dia} are systole and diastole CCA diameters, respectively; P_{sys} and P_{dia} are systolic and diastolic blood pressure levels.

Event-free survival was studied based on assessment of the time to occurrence of the combined endpoint, which included patient death (from all causes), clinically significant cardiac graft dysfunction requiring repeat HT, and clinically significant cardiac graft ischemia associated with transplant coronary artery disease (TCAD) with indications for coronary angioplasty.

Statistical processing of the study results was performed using the Wizard Pro software package (Version 1.9.49, MacOS). The Shapiro–Wilk test was used to check the normality of distribution of values. Student's t test was used to assess the reliability of differences in quantitative indicators that satisfied the normal distribution assumptions; in other situations, the Mann–Whitney U test was used. Differences in qualitative characteristics were assessed by constructing conjugation tables and their subsequent analysis using the chi-squared test. The Kaplan–Meier approach was used to evaluate event-free survival, and the log-rank method was employed to compare survival curves. In all statistical analytic methods used in the study, differences were considered reliable at $p < 0.05$.

RESULTS AND DISCUSSION

The predictive value of the CCA elasticity was examined in 101 heart recipients. Most patients had end-stage chronic heart failure, which was caused by dilated cardiomyopathy in 51 patients and ischemic cardiomyopathy in 33 patients. The patients included 80 males and 21 females; recipient mean age was 47.9 ± 1.9 years. The study included individuals not younger than 15 and not older than 78 years of age who survived 30 days after surgery.

Carotid ultrasound was performed on average 469.91 ± 280.65 days after HT. The median iCOMPL of the CCA in this heart recipient sample was 0.044 mm^2/mmHg. The recipients were divided into 2 subgroups depending on the value of elasticity index values above or below the median (0.044) (Table).

Patients with high elasticity (iCOMPL >0.044 mm^2/mmHg) did not differ on average from recipients with low iCOMPL in terms of age, sex, number of patients with a previous history of CHD, duration of post-HT follow-up. No significant differences were also found in common carotid artery intima-media thickness (CCA-IMT) and PWV, while the difference in iCOMPL was statistically significant ($p < 0.001$).

The overall follow-up was 3010.8 ± 280.0 days (126 to 8652 days, 95% CI: 2730.9–3290.8 days). During

Table

Comparative characteristics of heart recipients depending on the iCOMPL of the CCA

Indicator	iCOMPL <0.044 mm^2/mmHg	iCOMPL >0.044 mm^2/mmHg	Significance of differences (p)
Total	50	51	–
Number of females	12	9	0.432
Age	46.9 ± 3.5	46.8 ± 4.3	0.953
Number of patients with a history of coronary artery disease	16	17	0.268
Time after OHT until indicators are determined	532.8 ± 246.0	484.7 ± 125.5	0.729
CCA-IMT (mm)	0.76 ± 0.06	0.78 ± 0.04	0.689
PWV (m/s)	14.7 ± 1.6	13.3 ± 1.8	0.241
COMPL (m^2/mmHg)	0.029 ± 0.002	0.082 ± 0.013	**<0.001**

Note: CAD, coronary artery disease; OHT, orthotopic heart transplantation; CCA-IMT, common carotid artery intima-media thickness; PWV, pulse wave velocity; COMPL, common carotid artery elasticity index.

the follow-up, the combined endpoint (death, heart re-transplantation, and TCAD requiring coronary angioplasty) of the cardiac graft developed in 61 patients within 2754.0 ± 346.8 days (153 to 5226 days, 95% CI: 2407.3–3100.8 days).

In the recipient subgroup with a low estimated iCOMPL, mean follow-up was 2677.0 ± 402.1 days (95% CI: 2274.9–3079.1 days), 33 adverse events developed between 153 and 4,337 days, mean time-to-event was 2475.8 ± 479.9 days, 95% CI: 1995.8–2955.7 days.

In the subgroup with high estimated iCOMPL, mean follow-up was 3338.1 ± 381.7 days (95% CI: 2956.4–3719.8 days), 28 adverse events developed between 378 and 5.226 days, mean time-to-event was 3250.1 ± 647.5 days, 95% CI: 1995.8–2955.7 days).

Kaplan–Meier survival curves in subgroups with elasticity values greater and less than the distribution median are shown in the Figure.

Comparison of event-free survival curves revealed a significantly more favorable prognosis in heart recipients with estimated iCOMPL higher than 0.044 (mm^2/mmHg) ($p = 0.03$). At the same time, the study of the relationship between adverse event-free survival and age, sex, intima-media thickness, and pulse wave velocity did not reveal any significant dependence.

The findings of this study therefore suggest that a relatively low estimated iCOMPL of the CCA is a predictor of earlier development of adverse events in heart recipients, such as patient death, clinically significant heart graft dysfunction that required repeated HT, and clinically significant heart graft ischemia linked to TCAD with indications for coronary angioplasty, in heart recipients who do not exhibit morphological and immunohistochemical signs of heart graft rejection.

Previously, we had studied the properties of the index reflecting the degree of carotid arterial wall stiffness (iRIG), which was calculated based on ultrasonographic morphometric and Doppler parameters of the common carotid artery – CCA diameters in systole and diastole, peak systolic and end-diastolic velocity and blood flow acceleration time under the influence of systolic pulse wave [9]. That index, however, took longer to calculate even though it was designed to accurately reflect the true arterial wall stiffness. The elasticity index formula used in this work requires measurement of only two parameters – CCA diameters in systole and diastole, without the need for Doppler ultrasound of intravascular blood flow velocity parameters.

In previous studies, we have demonstrated that heart graft rejection is accompanied by changes in the elastic properties of the wall of the main arteries, in particular the CCA, and we established a vascular wall stiffness index linked to the risk of graft rejection [11]. Research has shown that, with effective rejection treatment, changes in the CCA elastic properties in rejection may be functional in nature and reversible [8].

The pathogenetic relationship between iCOMPL determined in this work with long-term prognosis may be down to the fact that arterial elasticity is dependent on both the tone of smooth muscle cells and the severity of remodeling, which is in turn characterized by the quantity and quality of connective tissue fibers, calcinosis, severity of inflammation, and cellular composition of the arterial wall. The endothelium plays an important role in regulating the vascular tone. Increased circulating blood volume, pulse wave asynchrony, sympathetic stimulation, activation of inflammation and action of proatherogenic factors are accompanied by endothelial dysfunction and, consequently, decreased arterial elasti-

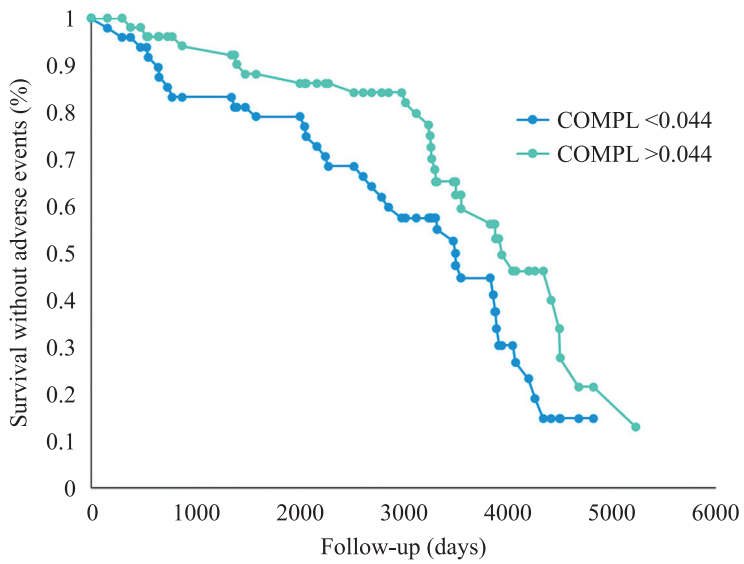

Fig. Kaplan–Meier event-free survival curves in subgroups of patients with different CCA wall elasticity (iCOMPL, vascular wall elasticity index)

city. In other words, the mechanisms leading to adverse events – cardiac graft cardiopathy and its complications, TCAD development and progression, atherothrombosis, irreversible cardiac graft dysfunction requiring retransplantation, and patient death – are, to varying degrees, directly or indirectly associated with decreased elastic properties of the wall of large arteries.

CONCLUSION

The iCOMPL of the CCA, which was developed and tested during the study, is a noninvasive and easily reproducible predictor of adverse events in the long-term post-HT period, which can be used in clinical practice for the purpose of risk stratification in heart recipients.

The authors declare no conflict of interest.

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BLOOD AND BONE MARROW CELL DISORDERS IN THE STAGES OF PROGRESSIVE DIABETES IN MICE

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Objective: to examine how the severity of tissue metabolic disorders affects the dynamics of the state of blood cells and bone marrow (BM) cells in patients with progressive diabetes mellitus (DM). **Materials and methods.** The genetic model of type 2 diabetes (T2DM) in db/db mutant mice (experimental group, n = 30) was used. Healthy mice of the same line – db/+m (n = 10) and line B10 (n = 5) served as control. The dynamics of laboratory and clinical parameters (blood glucose, glycosylated hemoglobin, body weight) and oxidative metabolism indicators in tissues were monitored FOR 6–6.5 months using Lasma-ST device. The state of blood cells (red blood cells, white blood cells, platelets) and BM cells were examined during the same period. Statistical processing of the results was done with preliminary use of the Shapiro–Wilk test; the significance of differences with the control was assessed using the parametric Student's t test, at $p < 0.05$. **Results.** In the development of T2DM, 3 stages of progressive metabolic disorders were identified: I – adaptation stage (1–2 months); II – progressive maladaptation stage (2.5–4.5 months); III – decompensation stage (from 5.0–6.5 months to death). It was found that in T2DM mice, blood content of red blood cells, Hb and leukocytes was reduced already in stages I–III; but in stage II and especially in stage III, there was increased platelet count and percentage of neutrophils, monocytes, eosinophils with a decrease in lymphocytes. A high percentage of live cells is preserved in the BM in stages I, II and early periods of stage III; in late periods of stage III, live cell percentages are frequently found to be low; in all periods of stage III, the total cell content in the BM is clearly reduced. **Conclusion.** Hematopoietic processes are inhibited in the BM as T2DM progresses. Individual assessment of the state of BM and its cells at the progressive stages of T2DM may be useful for prognostic purposes.

Keywords: diabetes mellitus, redox processes, blood cells, bone marrow cells.

INTRODUCTION

The prevalence of diabetes mellitus (DM) in the world is steadily increasing and has now become a pandemic [1]. Chronic course, severe vascular complications, early disability and high mortality among DM patients indicate the need to continue improving the therapy for this disease based on results from in-depth studies of pathogenetic mechanisms.

There are 2 most common types of DM – type 1 diabetes (T1DM) and type 2 diabetes (T2DM), which differ in terms of mechanisms of development and clinical manifestations at early stages of the disease.

Progressive hyperglycemia is a common clinical feature of T1DM and T2DM. It creates conditions for life-threatening complications in the body [2–4]. It has been shown that hyperglycemia has a damaging effect on various tissues of the body due to the toxicity of glycosylated proteins and lipoproteins accumulating in cells [5]. Among vital organs, the bone marrow (BM), which produces various types of blood cells (red blood cells, white blood cells, platelets) on a daily basis, turned out to be the most susceptible to the damaging effects of glycosylated proteins that accumulate in erythrocytes in the form of glycosylated hemoglobin [6]. As a result, red blood cells (RBC) change their functional properties (membrane potential decreases), and, along with white blood cells that produce proinflammatory cytokines [7], platelets play an active role in the development of life-threatening macro- and microvascular complications [8]. Other pathogenetic variables that emerge during the disease's progression promote disorders in the state of blood cells and BM in DM, particularly in T2DM. These include systemic inflammation against the background of developing immune dysfunction, oxidative stress and endoplasmic reticulum stress in cells, as well as disorders

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in the intestinal microbiome and barrier properties of the intestinal mucosa, etc., as a result of toxic damage to its cells [9–12]. Collectively, these factors aggravate metabolic disorders in the body by inhibiting redox processes in the cells of all organs and tissues, including blood and BM cells.

In recent years, as cell technologies have advanced, studies have been conducted on the feasibility of using bone marrow cells (BMCs) to treat metabolic disorders in T1DM and T2DM. These studies were based on the modern ideas that BM is not only the central organ of immunogenesis, but also the main regulator of the body's reparative regeneration processes [13]. However, it was discovered that autologous BM obtained from DM patients for induction therapy, even at early stages of the disease, have lower regulatory activity [14, 15] and are not always suitable for regenerative therapy [16] compared to BM from healthy allogeneic donors. We have not found any data on the state of BMCs at later, more severe stages of diabetes, when the patient undergoes not only drug therapy but also tissue (pancreatic islet cell transplantation) or organ (pancreas) transplantation. Meanwhile, it can be assumed that the outcomes of tissue and organ transplantation in patients with severe DM will be determined, among other things, by the state of their BMCs, their ability to adapt the body to the graft and support the graft viability in the recipient's body.

The aim of the present work is to study the dynamics of changes in the state of blood cells and BMCs depending on the severity of metabolic disorders in body tissues during the progressive course of diabetes on a genetic model of T2DM in mice.

MATERIALS AND METHODS

The dynamics of metabolic disorders, as well as changes in the state of blood cells and BMCs in T2DM were studied on mutant (homozygous) C57BL/KsJYLeprdb/+(B/Ks-Leprdb/+) – (db/db) mice, which carry a recessive gene – leptin receptor – Leprdb – (db) (linkage group 8, chromosome 4). The db gene in the homozygous state causes progressive DM, which is caused by a decrease in receptor-mediated sensitivity of body cells to endogenous insulin. The developing diabetes is similar to T2DM in humans and is characterized by cell degradation in pancreatic islets, but without deficiency of insulin production at early stages. There were a total of 30 mutant B/Ks-Leprdb/Leprdb (db/db) diabetic mice of both sexes used in the experiment (n = 30). Phenotypically healthy heterozygous mice of the same line – B/Ks-Leprdb/+ – (db/+m) (n = 10) and mice of non-diabetic line C57BL/10 – (B10) (n = 5) served as controls. So, the number of mice used in the experiment was 45 mice of the same initial age.

In these mice, changes in several functional indices developing in T2DM, which reflect the severity of the animal's clinical state, were studied dynamically over 6.0–6.5 months. Glucose and glycosylated hemoglobin (HbA1c) levels in the blood and body weight were measured, and the state of redox processes in the body tissues was assessed. Glucose levels were determined in fresh venous blood by photometric method on an Accu-Chek device (Switzerland), and the percentage of HbA1c was measured on an NycoCard READER device (Norway), which is designed for rapid *in vitro* determination of HbA1c by borate affinity analysis. The body weight of animals was determined using Mettler BD202 scale (Switzerland). Dynamic assessment of the state of redox processes was done noninvasively using a laser Doppler flowmeter, Lasma-ST [17]. This device allows measuring blood and lymph microcirculation in rodent tail tissues, determining in these tissues the activity level of mitochondrial coenzymes – NADN and FAD – and automatically calculating the oxidative metabolism index (OMI) based on the obtained results [17]. Determination of tissue microcirculation level, activity of mitochondrial coenzymes, oxidative metabolism index, as well as blood glucose levels in the course of animal life allowed us to identify 3 stages of metabolic disorders in T2DM mice (see the "Results" section of this paper). It was at these stages that we studied the dynamics of changes in the state of blood cells and bone marrow cells in T2DM mice.

In studying blood cells in T2DM, mixed (arterial-venous) blood was taken from cervical arteries and veins by decapitation of mice, preliminarily anesthetizing them with injection of Zoletil solution in saline at a dose of 40 mg/kg. Blood was collected in tubes with KZEDTA (tricalic salt of ethylenediaminetetraacetic acid). Hematologic parameters were evaluated – red blood cells (RBC, 10^{12}/L), hemoglobin (HGB, g/L), hematocrit (HCT, %), mean corpuscular volume (MCV, fl), mean corpuscular hemoglobin (MCH, pg), mean corpuscular hemoglobin concentration (MCHC, g/L), red cell distribution width (RDW-CV, %), red cell distribution width (RDW-SD, standard deviation, %), platelets (PLT, 10^9/L), relative platelet distribution width (PDW, %), plateletcrit (PCT, %), mean platelet volume (MPV, fl), white blood cells (WBC, 10^9/L), neutrophils (Neu, %), lymphocytes (Lymph, %), monocytes (Mono, %), Eosinophils (Eosi, %), basophils (Baso, %) were performed on an automatic hematology analyzer DYMIND VET DF50 (China) according to the manufacturer's guidelines. The data were presented as the average of three measurements.

BMCs were isolated from the femurs and tibias of mice using a standard protocol [18]. The isolated whole bones were cleared of muscles and ligaments, then the epiphyses were cut off and the bones were placed in 0.5 ml standard plastic centrifuge tubes with the bottom pre-pierced with a needle (G18–21). These tubes were placed inside 1.5 ml plastic centrifuge tubes and centrifuged for 10 seconds at 10,000g. The bone-purified BMCs sample was in the lower 1.5 mL tube after centrifugation.

Phosphatidylserine detection on the outer membrane of cells using labeled annexin V was used to assess the severity of BMCs apoptosis. The number of annexin-positive cells was assessed using the Annexin V-AF 488 Apoptosis Detection Kit (Lumiprobe, Russia) according to the standard flow cytometry protocol [19].

The obtained BMCs were suspended by pipetting in 100 µl of buffer at room temperature. From this suspension, so many cells were selected that their concentration in the reaction volume (100 µL) was $1 \times 10^5 \ldots 1 \times 10^6$ cells/mL. Annexin V-AF 488 was added to a concentration of 3 µg/mL and incubated for 15 minutes at room temperature. After that, 400 µL of chilled binding buffer was added.

Propidium iodide (PI) was used to assess cell membrane integrity. It was added to the samples before measurement on a flow cytometer to a concentration of 0.5–1 µg/mL.

Freshly prepared samples were analyzed on a BD FACSCalibur flow cytometer (Becton Dickson, USA) equipped with an argon laser (488 nm). Fluorescence emission (AF488, FITS) was recorded in a FL1 channel (515–545 nm) and in a propidium iodide FL2 range (620 nm). Between 15,000 and 25,000 events were accumulated for each sample. Data were collected using the CELLQuest program (Becton Dickson, USA). The data obtained in the pilot study were processed in the FlowJo program. The results were analyzed taking into account the guidelines outlined by Crowley et al. [19], without setting the target gate.

The numerical values of oxidative metabolism, glucose, HbA1c and body weight were statistically processed with preliminary use of the Shapiro–Wilk test on a small number of samples ($n < 5$) to demonstrate the normal distribution of data characterizing metabolism in individual periods. The significance of difference between the compared indicators was assessed using Student's t test (standard software package Microsoft Excel 2019) at $p < 0.05$.

RESULTS

The results of dynamic study of the clinical state of the animals during progressive development of T2DM and oxidative metabolism in their body tissues at the same age (time) periods are presented in Table 1 and Fig. 1. Table 1 shows that already after 1 month of life, the glucose and HbA1c levels of T2DM mice significantly increase, as does the body weight, compared to the controls. Continued examination of glucose and HbA1c levels in T2DM mice at 2, 4, and 6 months established further progressive increase. The body weight of T2DM mice at 2 and 4 months of life also continued to increase compared to controls, indicating obesity. However, starting at 5–6 months of age, the body weight of these mice became significantly lower than that of controls, and the animals acquired an emaciated appearance.

The characteristic clinical signs of T2DM, such as polydipsia, polyphagia and polyuria, became distinctly pronounced from 2 months after birth. On average, these mice drank 25.74 ± 1.18 ml of water per day, compared to 4.69 ± 0.35 ml in the control, $p < 0.05$; they ate 8.9 ± 0.29 g of feed compared to 3.74 ± 0.096 g in the control, $p < 0.05$ (control was briquette feed).

We identified the stages of increasing changes in the bodies of T2DM mice by dynamically measuring microcirculatory tissue indicators that characterize the state of redox processes in body tissues (Fig. 1) and comparing them to indicators of laboratory and clinical animal state (Table 1). It was found (Fig. 1) that at 1.0–1.5 months against the background of increasing hyperglycemia, the amplitude of the activity of coenzymes NADH and FAD increased, while OMI values (an oxidative metabolism indicator) decreased; however, when compared to the controls, the revealed changes in redox processes were not significant. This T2DM development period was recognized as stage I of T2DM and named the adaptation stage. Clinical signs of maladaptation appear at 2.0–2.5 months (significant increase in body weight, glycemia, HbA1c (glucose toxicity), polyuria, polyphagia) and last until 4.0–4.5 months. During this time span, the severity of impairment of redox process indicators progresses considerably (Fig. 1), although no visible consequences emerge yet. We defined stage II of T2DM as a period ranging from 2.0 to 4.5 months and dubbed it the progressive maladaptation stage. At the age

Table 1

Age dynamics of glucose content, HbA1c% and body weight in db/db, db/+m and B10 mice

Indicators of carbohydrate metabolism and body weight	Mouse lines		
	db/db (T2DM) Group 1 (n = 30)	db/+m (control) Group 2 (n = 10)	B10 (control) Group 3 (n = 5)
Age 1 month			
Glucose, mmol/L	10.3 ± 2.4*	5.4 ± 0.5	5.6 ± 0.3
HbAlc, %	4.9 ± 1.0*	3.5 ± 0.07	3.0 ± 0.08
Body weight, g	21 ± 2.5*	13 ± 1.2	15 ± 1.8
Age 2 months			
Glucose, mmol/L	18.7 ± 3.83*	5.8 ± 0.42	5.9 ± 0.03
HbAlc, %	7.9 ± 1.11*	3.6 ± 0.1	3.2 ± 0.13
Body weight, g	39 ± 2.37*	15 ± 2.69	18 ± 2.49
Age 4 months			
Glucose, mmol/L	25.5 ± 3.49*	4.6 ± 0.39	4.9 ± 0.69
HbAlc, %	8.6 ± 1.16*	3.7 ± 0.25	3.7 ± 0.22
Body weight, g	48 ± 2.68*	19 ± 2.26	21 ± 2.27
Age 6 months			
Glucose, mmol/L	27.4 ± 2.09*	5.7 ± 0.65	5.4 ± 0.38
HbAlc, %	8.9 ± 1.25*	3.9 ± 0.57	3.8 ± 0.49
Body weight, g	20 ± 2.35*	24 ± 1.80	27 ± 1.64

Note: *, $p < 0.05$ compared to control groups.

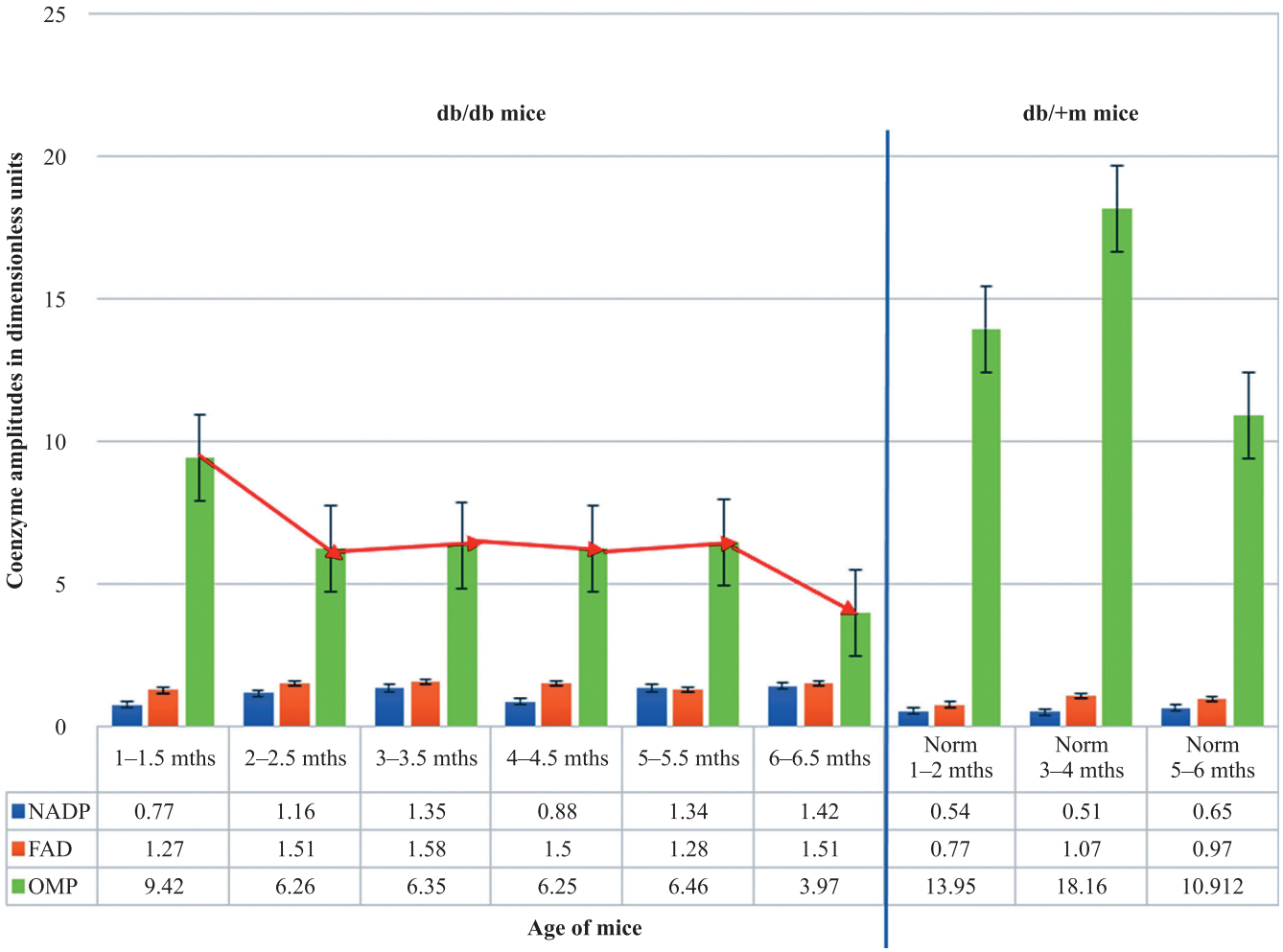
of 5.0–6.5 months, T2DM mice, on the background of worsening impairment of clinical parameters and indicators characterizing the state of redox processes in the body (NADN, FAD and OMI), with OMI reaching extremely low values – 3.97 ± 1.39 against 10.91 ± 2.04 in the control – at the same life stage (see Fig. 1), 30% of the animals developed late complications (skin maceration most often in the withers area), which persisted in the form of extensive wounds until the animals died (by 7–10 months). We defined this period (from 5.0–6.5 months until the animals died) as stage III of T2DM – the stage of decompensation of adaptation mechanisms with the development of deep tissue hypoxia, cell apoptosis and necrosis [8].

Having identified 3 clinical stages in the progression of metabolic disorders in T2DM, we proceeded to study the state of blood cells (RBCs, platelets and different types of WBCs) and BMCs, which produce them, since it is the state and functional properties of these cells that largely predetermine the adequacy of the course of redox processes in body tissues. Table 2 presents the results of a pilot study of the state of RBCs and platelets in healthy db/+m mice (control) and in db/db mice (T2DM model) at different stages of T2DM (abbreviations of the studied parameters are given in the "Materials and Methods" section).

Table 2 shows that already in the early life of T2DM mice (1.5–2.0 months, adaptation stage), their blood has a lower RBC content and reduced HGB level in RBCs compared to the control (db/+m mice). The increase in RBC at the decompensation stage is apparently a consequence of blood thickening on the background of polyuria.

In addition, T2DM mice showed a tendency towards increased MCV, RDW-SD and PLT, as well as decreased MCHC already at an early age. At the progressive maladaptation stage and decompensation stage, the same tendency towards an increase or decrease in some RBC characteristics was observed, which, apparently, indicates that structural alterations are emerging in these cells.

The PLT study clearly revealed a sharp increase in the number of these cells in blood at the stage of decompensation of redox processes and carbohydrate metabolism in the T2DM mice. In the study of WBC level (Table 3),



	1–1.5 mths	2–2.5 mths	3–3.5 mths	4–4.5 mths	5–5.5 mths	6–6.5 mths	Norm 1–2 mths	Norm 3–4 mths	Norm 5–6 mths
NADP	0.77	1.16	1.35	0.88	1.34	1.42	0.54	0.51	0.65
FAD	1.27	1.51	1.58	1.5	1.28	1.51	0.77	1.07	0.97
OMP	9.42	6.26	6.35	6.25	6.46	3.97	13.95	18.16	10.912

Fig. 1. Dynamics of microcirculatory and tissue parameters in db/db mice with DM and in db/+m mice without DM (normal) at different ages (the age of mice is indicated under the amplitudes of measured parameters: nicotinamide adenine dinucleotide phosphate (NADP), flavin adenine dinucleotide (FAD) and oxidative metabolism parameters (OMP) [17]

we also found a decrease in the total number of WBCs already at the early stages of life of the T2DM mice (1.5–2.0 months) compared to the control – 5.22×10^9/l *vs.* 9.92×10^9/l.

As the lifespan of the T2DM mice increased, the decrease in WBCs progressed, as seen by changes in the ratio of their individual populations. Early in the lives of the T2DM mice, the percentage of neutrophils (Neu), monocytes (Mono), and eosinophils (Eosi) increased, but the percentage of lymphocytes (Lymph) decreased. These changes intensified and became distinctly pronounced at the progressive maladaptation stages, especially during decompensation in the state of redox processes (Table 3).

The steady increase in platelet, neutrophil, monocyte and eosinophil count against a progressive decrease in lymphocyte count, as well as a sharp rise in the neutrophil-to-lymphocyte ratio suggest that systemic inflammatory response in T2DM is becoming more activated and that reparative processes are being inhibited, creating conditions for the development of micro- and macrovascular complications [25–27].

The detected decrease in the quantitative content of RBCs and WBCs already at early stages of life of T2DM mice in comparison with the control indicate that hematopoiesis processes were inhibited and that the state of BM responsible for hematopoiesis processes and regulation of homeostasis in the body of these mice needs to be assessed (Fig. 2).

Fig. 2 shows that during the development of T2DM, the percentage of large and medium-sized (proliferating) cells at stages I, II, and at early stage III decreases significantly compared to the control. In late (terminal) stage III of T2DM, the percentage of destroyed and small (nondividing) cells increases dramatically. Examination of the percentage of live and damaged cells in BM showed

Table 2

Results of dynamic study of the state of blood cells (red blood cells and platelets) in db/+m (control) and db/db (T2DM model) mice

Indicators studied	db/+m		db/db (T2DM)		
	1.5–2 mths.	3–4 mths.	1.5–2 mths. (adaptation period)	2.5–4.5 mths. (progressive maladaptation period)	5.0–6.0–6.5 mths. (decompensation period)
RBC, 10^{12}/L	8.68	8.36	7.5	7.73	8.12
HGB, g/L	157.5	154.5	132.25	147.3	155.7
HCT, %	40.75	40.35	39.15	40.8	43.88
MCV, fl	46.95	48.25	52.15	52.8	54.18
MCH, pg	18.1	18.45	17.6	19.07	19.18
MCHC, g/L	386	383	337.25	361	354.42
RDW-CV, %	15.35	13.65	18.42	17.53	17.4
RDW-SD, %	27.9	25.8	38.42	36.8	37.22
PLT, 10^9/L	881	732	898	753	1044.37
MPV, fl	6.7	6.7	6.85	6.33	6.7
PDW, %	6.5	6.75	6.22	7.2	8.07
PCT, %	0.59	0.49	0.61	0.48	0.69

Note: RBC, red blood cells; HGB, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW-CV, red cell distribution width; RDW-SD, red cell distribution width standard deviation; PLT, platelets; MPV; mean platelet volume; PDW, relative platelet distribution width; PCT, plateletcrit; fl, in femtoliters; pg, in picograms.

Table 3

Results of dynamic study of blood cells (white blood cells) in db/+m (control) and db/db (T2DM model) mice

Indicators studied	db/+m	db/db (T2DM)		
	2–4 mths.	1.5–2 mths. (adaptation stage)	2.5–4.5 mths. (progressive maladaptation stage)	5.0–6.5 mths. (decompensation stage)
WBC 10^9/л	9.92	5.22	4.37	3.32
Neu%	11.2	15.95	39.83	81.9
Lymph%	87.75	81.17	56.1	9.54
Mono%	0.65	1.22	2.3	6.14
Eosi%	0.3	1.57	1.67	2.33
Baso%	0.1	0.075	0.1	0.11
Neu/Lymph	0.13	0.20	0.71	8.58

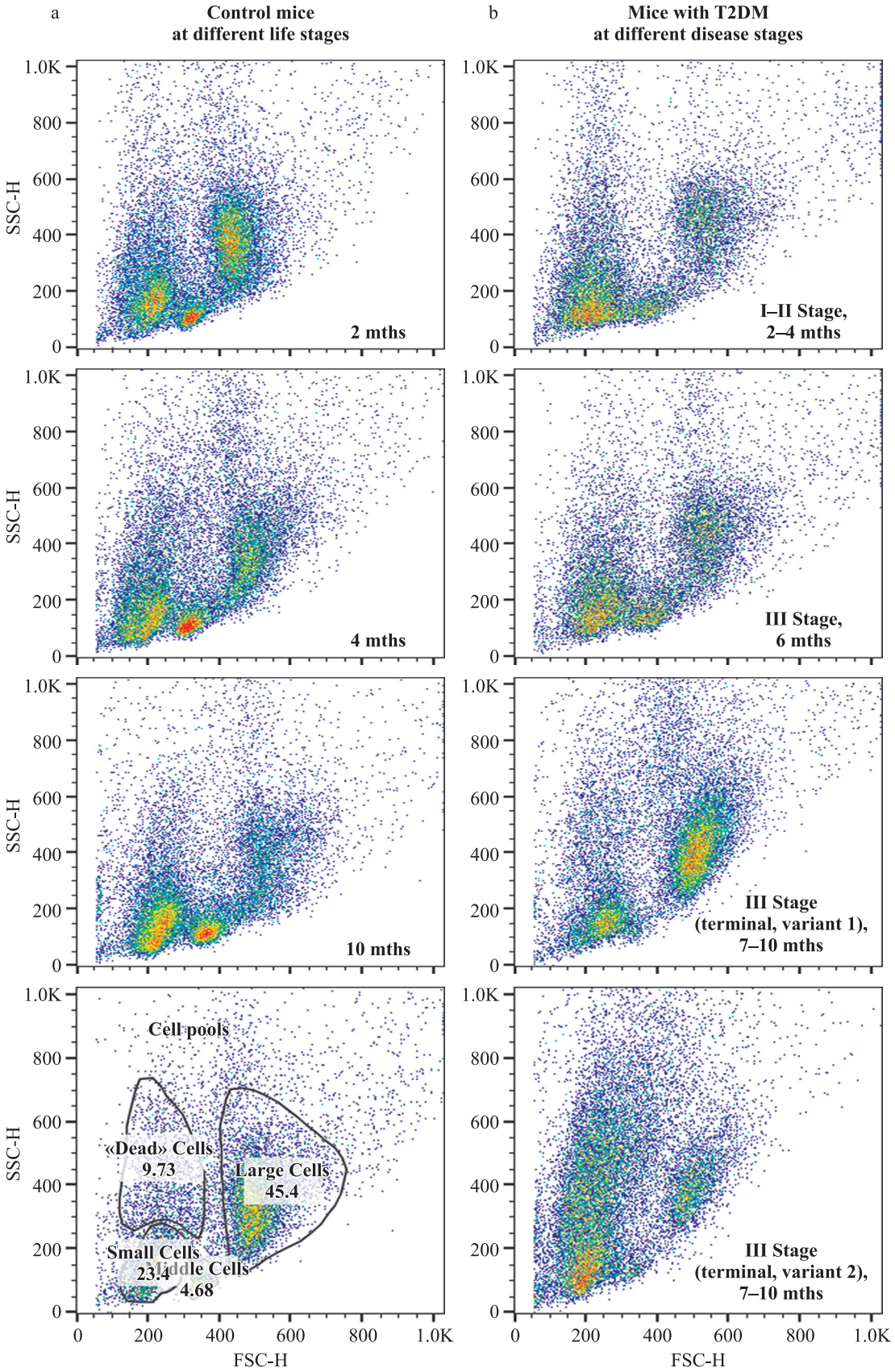


Fig. 2. Distribution of BM cells by size and texture in transmitted (FSC) and reflected (SSC) light in control mice (a) and mice with T2DM (b) at different life stages (disease stages). Bottom left shows the distribution of FSMs by population in control mice: small cells, middle cells, large cells, dead cells

(Fig. 3) that in healthy db/+m mice (control) at 2 months of age, the percentage of live cells averaged 68.95%, while cells in a state of necrosis, aponecrosis, and apoptosis averaged 31.05%. In db/db mice with T2DM at the same life span (adaptation period), the proportion of live cells was 71.35%, and cells in the state of necrosis, aponecrosis and apoptosis accounted for a total of 28.65%. At month 4 of life (period of developing maladaptation), as T2DM progressed, the percentage of living cells remained essentially unchanged, amounting to 70.5%; of the damaged cells, which made up 29.5%, the largest percentage (21%) were cells in the state of apoptosis.

In the early period of decompensation, which lasted for 5–6 months, however, we did not detect any deviations in the percentage of living and damaged cells in BM in contrast to the progressive maladaptation period (up to 4–4.5 months). Meanwhile, we noted a significant drop in the total count of BMCs during this period, as well as at later stages of clinical decompensation: BM gets depleted and it was necessary to collect BM from 2–3 tubular bones in order to study its cells.

At late stages of the decompensation period (7.0–9.5 months), the proportion of living cells in the BM of T2DM mice decreased significantly, reaching 30.4%; 69.6% of the cells were in necrosis, aponecrosis, or apoptosis, with aponecrosis accounting for the largest proportion of injured cells (44.7%). The BM of individual animals with T2DM showed a high percentage of living cells, even at late stages of decompensation, despite the fact that the total cell count was reduced when the cells were isolated from tubular bones (i.e., the bones contained a minimal amount of cellular material). Thus, in T2DM, as metabolic disorders worsen and redox processes become less efficient, the suppression of hematopoiesis processes also steadily increases in the BM, and cell necrosis, aponecrosis, and apoptosis intensify. These processes weaken the regulatory role and set the stage for the body to develop complications (skin maceration) and irreversible conditions. However, it is important to emphasize that even at the stage of clinical decompensation, at its early stage, and in some animals at the end stage, the BM cell pool is depleted, but not irreversibly damaged, indicating, in our opinion, the preservation of the regulatory and regenerative potential of BM in the body.

DISCUSSION

The issue of how clinical manifestations of diabetes and severe tissue metabolism disorders affect the state of blood cells and BMCs, particularly in the later stages of the disease, is still not well understood. Meanwhile, maintaining the mechanisms that keep the body in a state of homeostasis is crucial to the success of treatment and, most importantly, transplantation techniques (islet cell transplantation or pancreas transplantation) employed in the later stages of the disease. These primarily consist of BM, which is recognized as the central organ of immunogenesis, but also the main regulator of reparative regeneration in the body [13].

Regarding this, we set out to investigate in an experiment how the dynamics of changes in the condition of blood cells and BMCs are affected by the increasing disruption of carbohydrate and tissue metabolism in DM. We used a genetic T2DM model in 30 mutant db/db mice to investigate this issue. Healthy mice of the same line (db/+m) (n = 10) and mice of the B10 line (n = 5) were used as controls. In all these mice, laboratory and clinical parameters (blood glucose, HbA1c level, body weight, etc.), as well as the state of redox processes (by the level of microcirculation in tissues, amplitudes of coenzyme – NADH, FAD activity – and OMI, an indicator of oxidative metabolism) were monitored dynamically for 6.0–6.5 months from birth using the Lazma-ST apparatus [17].

T2DM was characterized by a progressive increase in hyperglycemia and glucotoxicity (increased HbA1c) until the end of the metabolic study period (Table 1). In the dynamics of body weight changes, two phases were identified: excessive weight gain over the course

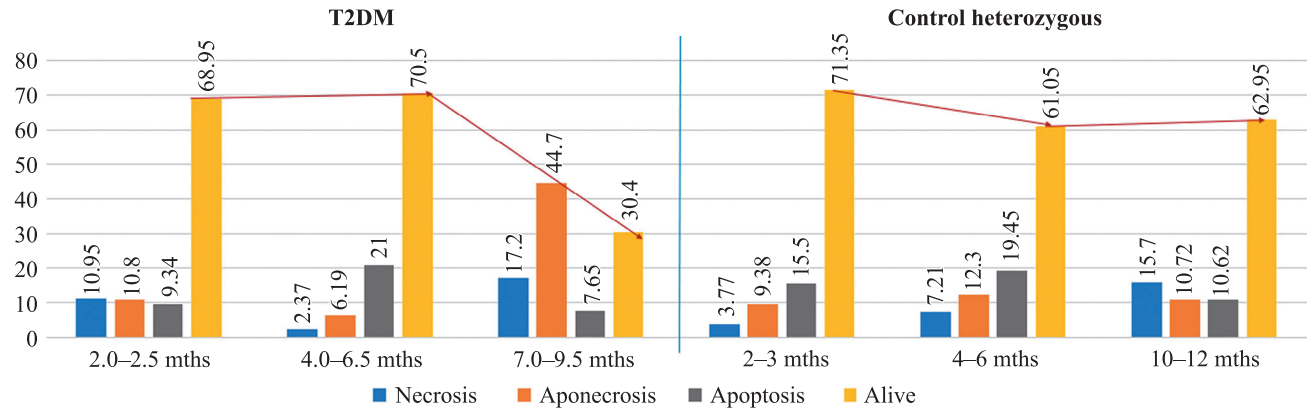


Fig. 3. Dynamics of changes in the state of bone marrow cells during the development of type 2 diabetes (in %)

of four months, followed by a fast decline over the next 5.0–6.5 months, all against the backdrop of persistent hyperglycemia and elevated HbA1c levels. This fact indicated profound metabolic disorders and the need for dynamic metabolic management.

In controlling the state of redox processes in the tissues of db/db mice, three stages of progressive T2DM were revealed (Fig. 1), which were characterized by a gradual increase in the severity of disorders in all parameters under study: I – adaptation stage (up to 2 months); II – progressive maladaptation stage (2.5–4.5 months); III – clinical decompensation stage (from 5–6.5 months to death of animals), at which vascular complications already appeared (skin maceration in 30% of mice). Additionally, we conducted a pilot study of the state of blood cells and BMCs in order to solve the task at the three stages. Against the background of hyperglycemia and elevated HbA1c, it was found that at stage I, the RBC count, HGB level, and WBC count all significantly decreased and stayed that way until the end of the observation (Table 2).

This fact can be explained by the fact that glycation of protein membranes of RBCs and other blood cells decreases their negative membrane potential, which accelerates aging and shortens the life span of blood cells [15]. In addition, a decrease in blood cell membrane potential in hyperglycemia promotes increased microviscosity, aggregation or adhesion of these cells, which first reduces their exit from the BM and then their production in the BM [16].

Patients with T2DM who experience prolonged episodes of hyperglycemia [17] and who already have microvascular complications [18] are observed to have decreased red blood cell count in the clinic. A decrease in erythrocyte count is also thought to be a result of erythropoietin production deficiencies in diabetic nephropathy patients, or of resistance to this hormone, as well as erythrocyte destruction that occurs in macro- and microangiopathies at advanced stages of T2DM [19].

However, we believe that in our experiments, the decrease in erythrocyte and leukocyte count already at stage I of T2DM in the model used, may be due to decreased membrane potential of cells as a result of early and accelerated glycation of the membrane proteins of these cells, caused by genetic features of the db/db mice. In stage II of T2DM, the persistent decrease in blood cell count may be due to all the above factors as well as to continuous rise in body weight.

Obesity is known to be accompanied by a state of chronic inflammation and a high level of circulating pro-inflammatory cytokines, which, having a long-term effect on the hematopoietic system and bone marrow niches, inhibit hematopoiesis processes in them [6]. The validity of this opinion is confirmed by our data on increased percentage of neutrophils, monocytes and eosinophils, and decreased lymphocytes in the blood (Table 3).

An increase in the neutrophil-to-lymphocyte ratio indicates activation of systemic inflammatory response in the body [20, 21] and even serves as a clinical predictor of worsening prognosis in the development of diabetic nephropathy [22, 23] and diabetic leg ulcers [24]. However, a cytometric analysis of BMC status, however, revealed that a significant portion (about 70%) of living cells remain in the BM at stages I, II, and even early stage III of T2DM (5–6 months) (Figs. 2 and 3). At the same time, the total cell count in the BM punctate always decreases significantly, even at early stage III. There is also always a significant drop in the total BMC count at the late progressive stage III of T2DM (7.0–9.5 months), a significant drop in the pool of living cells (up to 30.4%) and a high proportion of damaged cells (up to 69.6%); however, at late periods of stage III T2DM, a high percentage of living cells may also be retained in some animals. According to these findings, there are still T2DM animals, whose BMCs are resistant to the damaging effects of hyperglycemia and growing hypoxia, even at the decompensation stage of clinical and metabolic parameters (both at early and late stages). The ability of animals to contribute to the regulation and maintenance of homeostasis in the body is evident from the high percentage of living cells in their BM at the terminal stage of T2DM; their detection in the BM at the decompensation stage enables the prediction of a higher efficacy of therapeutic measures. A similar opinion is held by Gautier et al. (2015) [30], who consider it reasonable to predict the effectiveness of organ (liver) transplantation operations through preliminary measurement of the level of bone marrow CD34+ cells in peripheral blood, which characterizes the regenerative potential of cells in all body tissues [31].

CONCLUSION

1. In progressive T2DM in db/db mice, three stages are revealed, which differ in terms of degree of increase in the severity of redox processes and metabolic parameters: stage I (adaptation stage, at 1–2 months of life); stage II (progressive maladaptation stage, at 2.5–4.5 months of life); stage III (decompensation stage, from 5.0–6.5 months and until the animal dies).
2. Progressive T2DM occurs against the background of increasing hyperglycemia, elevated erythrocyte HbA1c levels, increased body weight at stages I and II and decreased body weight at stage III. These changes in clinical parameters occur against the background of a gradual decrease in the efficiency of redox processes (increased amplitudes of coenzymes NADH and FAD, and decreased OMI), especially pronounced at stage III.
3. In a pilot study of blood cell status in T2DM mice demonstrated a decrease in erythrocytes, Hb and leukocytes already at stage I. This persists at stages II and III. At stage II and especially at stage III, there is

a sharp increase in platelet count and percentage of neutrophils, monocytes and eosinophils, a decrease in lymphocyte count, as well as increased neutrophil-to-lymphocyte ratio, which indicate a systemic inflammatory response.

4. At stages I, II, and early stage III (5–6 months), the percentage of living and damaged cells in the BM of T2DM mice remains at the initial values; at late stage III (7.0–9.5 months), the percentage of living cells in the BM often decreases sharply and the percentage of damaged cells increases; at all periods of stage III, a decrease in the total cell count in the BM samples is diagnosed. Therapeutic interventions may be more successful in these animals if a significant proportion of living cells are preserved in the BM at both the early and late stages of the decompensation stage.
5. Individual assessment of blood cells and BMCs in progressive T2DM may be useful for prognostic purposes.

The research was partially conducted within the framework of the state assignment on the topic: "Assessment of the body's adaptive responses to physicochemical and environmental factors" (№ FGFU-2022-0010).

The authors declare no conflict of interest.

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GALECTIN-3 IN RECIPIENTS WITH KIDNEY GRAFT DYSFUNCTION: ANALYSIS OF PREDICTIVE SIGNIFICANCE

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One of the most pressing issues in contemporary transplantology is the ongoing search for less invasive methods that would identify potential complications that recipients of solid organ transplants may encounter. Profibrogenic factor galectin-3 (Gal-3) is a potential marker of such complications. It is presumed that it may be involved in regulatory processes in both physiological and pathological conditions; Gal-3 is of particular importance in diseases associated with chronic inflammation and fibrosis. **Objective:** to assess the predictive significance of Gal-3, determined in the recipients' serum, in the pathology of a transplanted kidney. **Materials and methods.** The study included 138 kidney recipients aged from 5 to 68 years and a group of healthy individuals (n = 11). Recipients' serum Gal-3 levels were measured by immunoenzymatic method. **Results.** Among the kidney recipients, 91 patients had kidney graft dysfunction according to laboratory and clinical data, which served as an indication to perform a graft biopsy with morphologic examination of the samples. In kidney recipients, Gal-3 levels were significantly different and higher than in healthy individuals, p = 0.017; it did not correlate with most blood test parameters, but there was an inverse correlation with graft glomerular filtration rate (GFR) (r = –0.174; p = 0.043). Recipients' Gal-3 levels were independent of their tacrolimus blood levels. Kidney recipients with graft dysfunction had considerably higher Gal-3 levels (p = 0.0003) compared to those without. Comparative analysis significantly showed higher Gal-3 concentrations in recipients with acute cellular rejection (ACR, p = 0.005), antibody-mediated rejection (AMR, p = 0.016) and calcineurin inhibitor (CNI) nephrotoxicity (p = 0.006) compared to recipients without dysfunction. Recipients with signs of CNI nephrotoxicity tended to have higher Gal-3 levels when compared to recipients with graft dysfunction of other etiology (p = 0.08). Kidney recipients with Gal-3 levels above the calculated threshold value of 7.63 ng/mL had a 2.89-fold higher risk of developing chronic graft dysfunction and/or requiring hemodialysis compared with the rest of the kidney recipients (RR = 2.89 ± 0.46 [95% CI 1.17–7.11]), with 76.2% sensitivity and 56.1% specificity of the test. **Conclusion.** The threshold serum Gal-3 level in kidney recipients can be considered a predictor of an unfavorable graft outcome (chronic graft dysfunction and/or a need for renal replacement therapy).

Keywords: galectin-3, kidney transplantation, graft disease, non-invasive diagnosis.

INTRODUCTION

Chronic kidney disease (CKD) is an irreversible and progressive disease that leads to end-stage renal disease and cardiovascular complications, which cause significant mortality in the population [1]. Patients with end-stage CKD require renal replacement therapy through hemodialysis, peritoneal dialysis or kidney transplantation. Although the mortality rate in dialysis patients has decreased in recent decades [2, 3], it still remains higher compared to the general population [4]. Kidney transplantation (KT) is the most desired treatment for end-stage CKD because it achieves a higher survival rate and quality of life, and reduces cardiovascular complications. In addition, KT has a lower cost than dialysis procedures [5–7].

Puncture biopsy of the graft is currently used to diagnose and confirm post-transplant complications in kidney recipients. Clinical signs of kidney graft dysfunction that have already developed due to immune damage or another injury are indications for an unscheduled biopsy. In addition, there is a risk of taking a non-diagnostic section of the transplanted kidney tissue for examination. It is evident that with early minimally invasive diagnosis, immunosuppressive therapy can be corrected early, preventing the development of kidney graft dysfunction or reducing its severity.

In this regard, in recent years, research has been actively conducted in the search for personalized methods of minimally invasive diagnostics of post-transplant complications based on analysis of the levels of molecular and genetic biomarkers, as well as their combinations

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[8]. The minimally invasive technology for biomarker quantification is based on measuring their concentration in blood and other biological media. Such biomarkers include profibrogenic factor galectin-3 (Gal-3), which has multiple effects in physiological and pathological processes. Gal-3 is a beta-galactoside-binding protein with a unique structure of polypeptide domains that allows it to interact with proteins in carbohydrate-dependent and -independent ways.

Gal-3 stimulates the chemotaxis of macrophages and monocytes, adhesion of neutrophils and activation of proinflammatory factors. Gal-3 has been shown to play a role in chronic inflammatory diseases and tissue fibrosis [9]. There is evidence of a connection between changes in serum Gal-3 levels in solid organ recipients: heart and lungs [10, 11], liver [12], kidneys [13], with the development of post-transplant complications. Assessing Gal-3 levels in recipients may be useful for improving the methods for early diagnosis of transplant pathology.

The aim of the present work is to evaluate the predictive significance of serum Gal-3 levels in recipients, in the pathology of a transplanted kidney.

MATERIALS AND METHODS

The study included 138 adult kidney recipients who underwent related kidney allotransplantation (RKAT) or cadaveric kidney allotransplantation (CKAT) from 1999 to 2022 at Shumakov National Medical Research Center of Transplantology and Artificial Organs.

The selected recipients included 91 with signs of graft dysfunction that required unscheduled puncture biopsy and 47 without signs of graft dysfunction. The dysfunction criteria were considered to be elevated creatinine and urea levels, as well as proteinuria. The comparison group consisted of 11 healthy individuals selected randomly and not significantly different in terms of age and gender from the kidney recipients. All recipients underwent routine examinations following KT, in accordance with the Shumakov Center's patient management protocol and the National Clinical Guidelines of the Russian Transplant Society. These examinations included a clinical evaluation, full blood count, biochemical blood tests and urine tests, measurement of blood tacrolimus levels, graft biopsy, and measurement of glomerular filtration rate (GFR).

Gal-3 levels were determined in venous blood serum. For this purpose, blood samples collected in disposable tubes were centrifuged, after which the serum was frozen and stored at -20 °C. Gal-3 concentration was measured by enzyme-linked immunosorbent assay using a specific reagent kit (Human Gal-3 ELISA Kit, RayBio®, USA) according to instructions. Blood samples for Gal-3 level analysis were collected on the day of biopsy and other routine laboratory tests (full blood count, biochemical blood tests, and general urinalysis).

All kidney recipients received standard triple-combination immunosuppressive therapy, including a combination of calcineurin inhibitors (tacrolimus, less frequently cyclosporine) in combination with mycophenolate and corticosteroids, and additional drug therapy as indicated.

The indication for puncture biopsy was renal graft dysfunction presenting as increased blood creatinine levels, either alone or in combination with proteinuria, as well as a marked decrease in GFR. The pathology of the transplanted kidney was verified on the basis of the morphological studies of the biopsy material according to the Banff classification. Sections of the obtained samples were stained with hematoxylin, eosin, Masson's trichrome and Periodic acid-Schiff (PAS).

The following pathology variants were identified: acute tubular necrosis in the early post-transplant period (ATN), acute cellular rejection (ACR), antibody mediated rejection (AMR), nephrosclerosis with signs of calcineurin inhibitor (CNI) nephrotoxicity, not associated with immune response (CNI nephrosclerosis), and recurrent glomerulonephritis of the transplant (chronic glomerulonephritis). Given the morphological study results, the recipients' immunosuppressive therapy was adjusted.

The development of chronic dysfunction against the backdrop of the ineffectiveness of the current therapy, or the need for renal replacement therapy (hemodialysis or peritoneal dialysis) in conjunction with minimizing immunosuppressive therapy while waiting for re-transplantation, were deemed criteria for an unfavorable outcome of graft condition.

The graft GFR was calculated using the CKD-EPI formula, which takes into account race, sex, age, and serum creatinine level.

Nonparametric statistics methods – Mann–Whitney U test and Spearman correlation – were used for comparative analysis of independent variables. Group differences were considered reliable at $p < 0.05$. The predictive significance of Gal-3 level was evaluated using ROC analysis. The optimal threshold level for predicting high risk of adverse KT outcome was determined using the Youden's index. The main diagnostic characteristics of the test were evaluated: relative risk (RR), 95% confidence interval (CI) limits, sensitivity (Se), and specificity (Sp). Software package Statistica v.13.0, StatSoftInc (USA) was used for statistical processing of the obtained data.

RESULTS

The study included 138 kidney recipients aged 5 to 68 years, among whom the number of men 68 (49.3%) and women 70 (50.7%) did not differ significantly.

The major proportion of patients (74.7%) underwent CKAT and the remaining 25.3% received a living RKAT. The follow-up period of the recipients ranged from 2 to 4,748 days (median, 325 days); 75% of the patients were examined in the long term (more than 1 month from the

date of transplantation). The main characteristics of the recipient group are presented in Table 1.

Serum Gal-3 levels of the subjects included in the study varied within a wide range of 5.8 [1.9; 17.8] ng/mL, did not differ significantly between men and women (p = 0.77), and did not correlate with age (r = –0.15; p = 0.14).

Gal-3 levels in kidney recipients was significantly different and higher than in healthy individuals, p = 0.017. Comparative analysis of Gal-3 concentration in those who received related kidney and a kidney from a postmortem donor revealed no significant differences (p = 0.083).

There was no significant correlation between Gal-3 levels and post-transplant period (days) (r = 0.125; p = 0.24); There were no significant differences in Gal-3 level in kidney recipients in the early (<30 days) and late (>30 days) post-transplant period (p = 0.57).

Table 1

Basic characteristics of kidney recipients and healthy subjects included in the study

Parameter		Kidney recipients	Healthy subjects
Number (n)		138	11
Gender (n (%)):	Male	68 (49.3%)	6 (55%)
	Female	70 (50.7%)	5 (45%)
Age (years):	Range Median [Interquartile range]	5 to 68 37 [26; 48]	10 to 64 44 [29; 54]
Type of transplant (n (%)):	Deceased donor (CKAT)	101 (73%)	–
	Living-related donor (RKAT)	37 (27%)	
Graft function (n (%)):	Normal function	47 (34%)	–
	Signs of graft dysfunction	91 (66%)	
Follow-up period (days):	Range Median [Interquartile range]	2 to 4748 325 [39; 1448]	–
Post-transplant period (n (%)):	Early (≤1 month)	34 (25%)	–
	Late (>1 month)	104 (75%)	
Gal-3 level (ng/mL):	Median [Interquartile range]	7.6 [1.9; 24.1]	2.75 [1.64; 3.11]

Table 2

Correlation of Galectin-3 levels with complete blood count, biochemical tests and urinalysis indicators in kidney recipients

Indicator	Spearman's rank correlation (r)	Significance level (p)
Complete blood count		
Hemoglobin (g/L)	r = –0.023	p = 0.870
White blood cells (10^9/L)	r = –0.191	p = 0.185
Platelets (10^9/L)	r = –0.164	p = 0.249
Blood chemistry test		
Total protein (g/L)	r = –0.021	p = 0.083
Creatinine (μmol/L)	**r = 0.179**	**p = 0.039**
Urea (mmol/L)	r = 0.169	p = 0.051
ALT (U/L)	r = 0.069	p = 0.476
AST (U/L)	r = –1.737	p = 0.083
Special blood test		
GFR (mL/min/1.73 m^2)	**r = –0.174**	**p = 0.043**
Tacrolimus (ng/mL)	r = –0.122	p = 0.183
Urinalysis		
Red blood cells (in the field of view)	**r = 0.176**	**p = 0.048**
White blood cells (in the field of view)	r = 0.132	p = 0.139
Proteinuria (g/L)	r = 0.002	p = 0.982

The relationship between Gal-3 concentration and the main indicators of complete blood count, biochemical tests and urinalysis was studied (Table 2).

Correlation analysis showed that Gal-3 level did not correlate with most blood test parameters, but it did correlate directly with creatinine level (r = 0.179; p = 0.039) and inversely with graft GFR (r = –0.174; p = 0.043). Recipients' tacrolimus concentrations did not affect Gal-3 levels.

Evaluation of the relationship between serum Gal-3 levels and urinalysis parameters showed a significant direct correlation with red blood cell count (r = 0.176; p = 0.048).

Out of all 138 recipients included in the study, 91 were categorized as "with graft dysfunction" and 47 were designated as "with normal function" based on laboratory and clinical data. Comparative analysis of the values of laboratory parameters in recipients with and without graft dysfunction is shown in Table 3.

Kidney recipients with graft dysfunction had significantly higher levels of creatinine and urea, GFR and proteinuria (p < 0.00001) compared to those without.

Comparative analysis of serum Gal-3 levels in these groups also showed significant differences (p = 0.0003).

A comparative analysis of Gal-3 levels in the blood of kidney recipients with and without graft dysfunction of different nature was carried out (Fig. 1).

It was found that in recipients with ACR (n = 29), AMR (n = 35) and CNI nephrosclerosis (n = 10), Gal-3 levels were significantly higher than in recipients with normal graft function (p = 0.005, p = 0.016 and p = 0.006 respectively).

There were no significant differences in Gal-3 levels in acute tubular necrosis (n = 11) and recurrent glomerulonephritis (n = 6) compared to recipients without graft dysfunction (p = 0.056 and p = 0.083, respectively).

No significant differences were found in a comparative analysis of Gal-3 levels in kidney recipients with graft dysfunction depending on the nature of the pathology. This may be due to the small patient sample size. On the other hand, receivers exhibiting signs of CNI nephrotoxicity tended to have higher Gal-3 levels than recipients experiencing graft dysfunction of other etiology (Fig. 2).

Table 3

Comparative analysis of laboratory parameters in kidney recipients with and without graft dysfunction

Indicator	Normal function	Graft dysfunction	Significance level
Creatinine (μmol/L)	85.3 [69.9; 97.83]	214.9 [151.7; 363.8]	**p < 0.00001**
Urea (mmol/L)	7.4 [5.9; 8.5]	18.52 [12.8; 26.2]	**p < 0.00001**
Proteinuria (g/L)	0.04 [0.03; 0.19]	0.14 [0.04; 0.40]	**p < 0.00001**
GFR (mL/min)	81.5 [70.23; 102.9]	26.34 [14.3; 43.7]	**p < 0.00001**
Gal-3 (ng/mL)	2.3 [0.06; 14.4]	8.75 [3.5; 28.5]	**p = 0.0003**

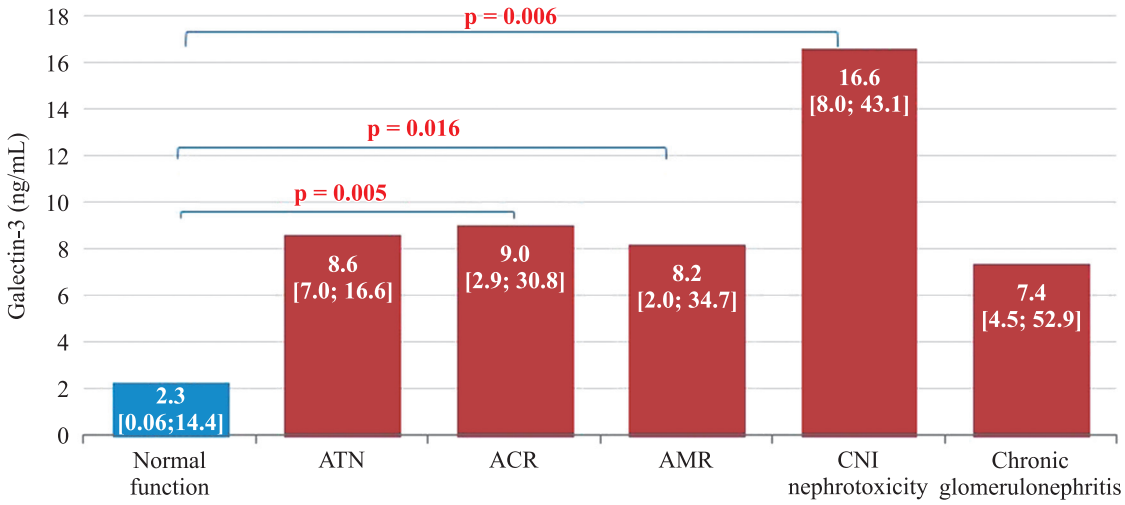

Fig. 1. Comparative analysis of Serum galectin-3 levels in kidney recipients with and without graft dysfunction of various natures. ATN, acute tubular necrosis; ACR, acute cellular rejection; AMR, antibody-mediated rejection; calcineurin inhibitor (CNI) nephrotoxicity

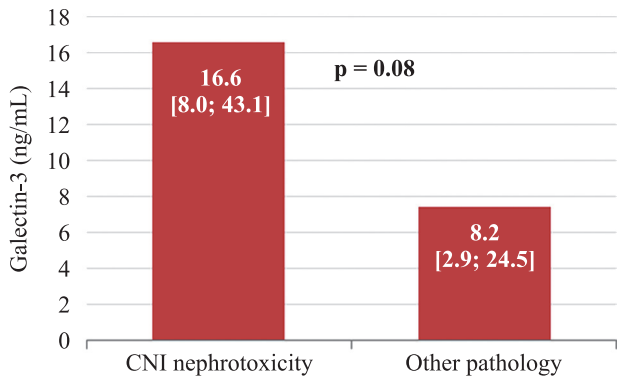

Fig. 2. Comparative analysis of galectin-3 levels in kidney recipients with signs of calcineurin inhibitor nephrotoxicity and other lesions

At the same time, in the level of classical renal function parameters (creatinine, urea, proteinuria and GFR), there was no tendency for difference in the recipients with signs of CNI nephrotoxicity compared to recipients with graft dysfunction of other etiology (Table 4).

Among kidney recipients in whom graft dysfunction was verified, 21 patients at long-term follow-up (13.6 [1.2; 48.3] months) developed an unfavorable graft outcome (chronic graft dysfunction and/or need for hemodialysis), while 57 patients had preserved graft function.

A comparative analysis of serum Gal-3 levels in kidney recipients and GFR, determined six months (5.4

Table 4

Comparative analysis of laboratory parameters of renal graft function in kidney recipients with signs of calcineurin inhibitor nephrotoxicity and other lesions

Indicator	CNI nephrotoxicity	Other pathology	Significance level
Creatinine (μmol/L)	186.6 [142; 259]	212.3 [149.1; 356.4]	p = 0.56
Urea (mmol/L)	16 [13.1; 20.7]	18.5 [12.5; 27.4]	p = 0.66
Proteinuria (g/L)	0.08 [0.04; 0.34]	0.14 [0.04; 0.40]	p = 0.53
GFR (mL/min)	31.4 [15.1; 38.7]	26.3 [14.3; 44.7]	p = 0.88

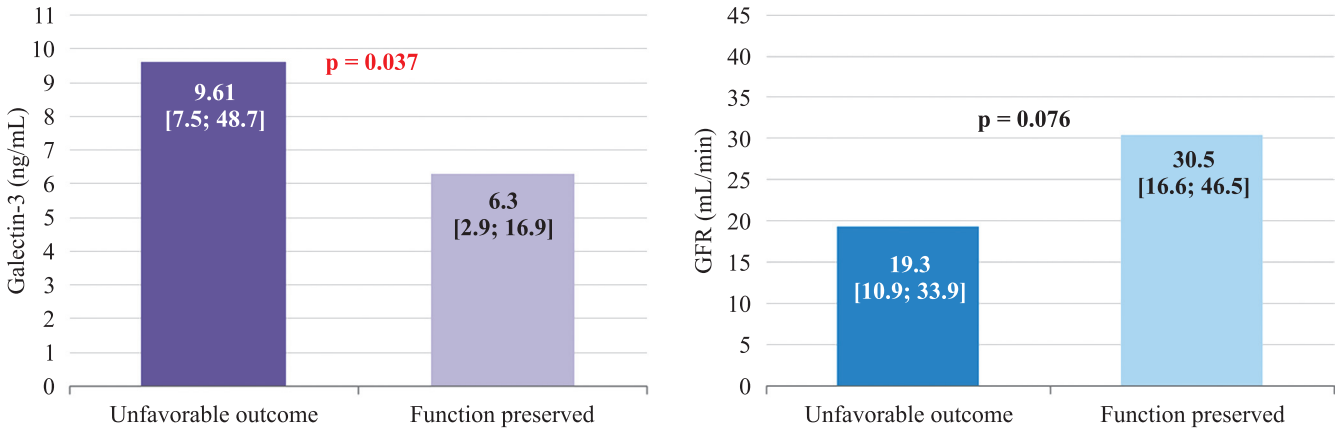

Fig. 3. Comparative analysis of baseline galectin-3 and GFR levels in kidney recipients with and without unfavorable graft outcome

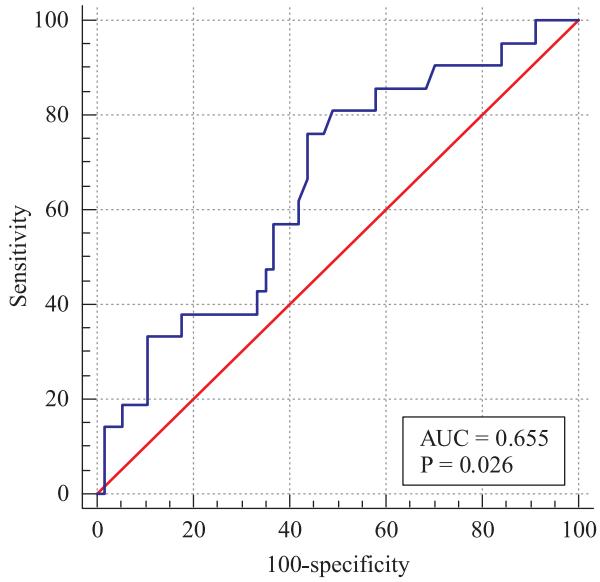

Fig. 4. ROC curve of serum galectin-3 levels in kidney recipients with unfavorable graft outcome

Table 5

Comparative analysis of kidney graft outcome and galectin-3 levels

Outcome	Recipients with Gal-3 levels >7.63 ng/mL (n = 41)	Recipients with Gal-3 levels ≤7.63 ng/mL (n = 37)	Significance (p)
Unfavorable	16 (39%)	5 (14%)	**0.021**
Favorable	25 (61%)	32 (86%)	

[1.8; 8.3] months) before the onset of an unfavorable graft outcome, was performed with the same indicators in patients with preserved graft function (Fig. 3).

It was found that the initial serum Gal-3 concentrations in kidney recipients were significantly higher in patients with chronic graft dysfunction and/or need for hemodialysis compared to recipients without it (p = 0.037). Baseline GFR scores did not differ between these recipient groups.

An assessment of the predictive significance of serum Gal-3 level in kidney recipients in relation to the development of unfavorable post-transplant outcome is presented in Fig. 4.

The area under the ROC curve was 0.655 ± 0.069 [95% CI 0.538–0.759] and was significantly (p = 0.026) different from 0.5.

The threshold serum Gal-3 level in kidney recipients, significant for predicting adverse graft outcome, was 7.63 ng/mL (Table 5).

Among 41 kidney recipients with Gal-3 level >7.63 ng/mL, 16 (39%) had an unfavorable kidney transplant outcome, which is significantly different from the group of recipients with Gal-3 ≤7.63 ng/mL, in which 5 (14%) out of 37 recipients had unfavorable outcome (p = 0.021).

Analysis of the predictive characteristics of Gal-3 level in relation to graft outcome in kidney recipients showed that in kidney recipients with Gal-3 level exceeding the threshold 7.63 ng/ml, the risk of developing

chronic graft dysfunction and/or requiring hemodialysis was 2.89 times higher than in the rest of kidney recipients with graft dysfunction (RR = 2.89 ± 0.46 [95% CI 1.17–7.11]), with a 76.2% sensitivity and 56.1% specificity.

DISCUSSION

The development in recent years of innovative approaches to the diagnosis and prediction of post-transplant complications has become possible due to active research in the field of biochemistry, immunology and genetics. Mechanisms of interaction between the recipient's body and the donor organ include a wide range of immunological responses that can lead to graft rejection [14]. After transplantation, recipients are still likely to develop ACR, AMR and chronic rejection, which lead to accelerated formation of graft fibrosis and graft dysfunction [15].

In this regard, early detection of post-transplant complications is of key importance for long-term graft functioning [16]. Carrying out a transplant puncture biopsy to verify the pathology of the transplanted kidney is not always informative due to the risk of sampling a tissue area without morphological signs of changes. In addition, laboratory data on declining renal function typically serve as an indication for a biopsy and already point to a chronic pathological process in the kidney graft [17]. The use of minimally invasive laboratory diagnostic methods in kidney recipients should improve the efficiency of detecting graft pathology at an early stage of development.

A large number of studies show that Gal-3 plays an important role in the development of various pathological processes, including inflammation, immune cell infiltration and fibrosis [18, 19]. This thesis is supported by research conducted on two animal groups by Dang et al. They found that mice with a genetic defect in Gal-3 in their kidney transplant had less tubular injury, moderate fibrosis and lower immune cell infiltration in comparison to the group of normal animals, in which characteristic changes in the transplant were determined in the form of renal tubular atrophy, as well as Gal-3 upregulation in tissues and blood plasma [13].

Another study showed that pharmacological suppression (modified citrus pectin) or genetic switching off of Gal-3 inhibited the upregulation of Gal-3 linked to fibrosis and renal dysfunction in models of experimental hyperaldosteronism [20].

In our study, it was shown that there was no correlation between Gal-3 levels and most laboratory blood test parameters, which suggests the independent diagnostic potential of Gal-3. At the same time, there was an inverse correlation between serum Gal-3 levels in the kidney recipients and transplant GFR ($r = –0.174$; $p = 0.043$).

These findings are consistent with those of Alam M.L. et al., who found that higher Gal-3 levels were associated with CKD progression and lower GFR <15 mL/min [21]. A study by Hsu B.G. et al. discovered an inverse correlation between serum Gal-3 level in CKD patients and GFR. In addition, it was suggested that Gal-3 is a potential modulator of endothelial function and can be used as a biomarker to diagnose endothelial dysfunction in patients with kidney injury [22].

CNIs occupy a special place among immunosuppressive drugs that prevent transplant rejection. The introduction of this group of drugs into daily clinical practice has improved survival rates and quality of life of solid organ recipients. However, the need for lifelong CNIs leads to a number of side effects, among which the nephrotoxic effect with the development of CNI nephrosclerosis is of particular importance in kidney transplant recipients [23]. In this study, no significant differences were found in a comparative analysis of Gal-3 levels in kidney recipients with graft dysfunction depending on the nature of the pathology. However, there was a tendency for higher Gal-3 levels in recipients with signs of CNI nephrotoxicity in comparison with recipients with graft dysfunction of other etiology ($p = 0.08$). This finding may be associated with fibrosis and functional remodeling of the graft in this cohort of kidney recipients.

In a study by Ou S.M. et al., it was shown that higher plasma Gal-3 levels in CKD were associated with interstitial fibrosis, tubular atrophy and vascular intima fibrosis. When evaluating RNA sequencing results, the authors showed an upregulation of Gal-3 in biopsy samples of fibrotic kidney [24].

To be able to predict kidney transplant survival, it is necessary to develop minimally invasive diagnostic methods using biomarkers to predict long-term transplant outcome. The present study evaluated the predictive significance of serum Gal-3 levels in recipients with kidney graft dysfunction in relation to the development of an unfavorable outcome (chronic graft dysfunction and/or need for dialysis). It was found that kidney recipients with Gal-3 levels exceeding the threshold value of 7.63 ng/mL have almost three times higher risk of developing chronic graft dysfunction and/or a need for dialysis compared to other kidney recipients, with a 76.2% sensitivity and 56.1% specificity. The insufficiently high specificity of the test is obviously down to the inclusion of only kidney recipients with existing graft dysfunction signs in the study group.

The threshold serum Gal-3 level in kidney recipients determined in this work can be considered as an additional test, specifically as a predictor of poor outcome of kidney transplant dysfunction, and be useful for early modification of immunosuppressive therapy and/or performance of unscheduled biopsy.

The study was conducted within the framework of state assignment.

The authors declare no conflict of interest.

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HIGH INCIDENCE OF RARE *TGFB1* HAPLOTYPES IN CHILDREN WITH BILIARY ATRESIA

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Objective: to evaluate the occurrence of single nucleotide polymorphisms (SNPs) in transforming growth factor beta 1 (*TGFB1*) – rs1800469, rs1800470, rs1800471 – and their haplotypes in children with biliary atresia (BA). **Materials and methods.** We studied 106 pediatric liver recipients aged 4 to 150 (median 8) months, of whom 44 were boys, and 199 healthy individuals aged 32.7 ± 9.6 years, of whom 79 were boys. The indication for pediatric liver transplantation was BA. Genomic DNA was isolated from peripheral blood using a commercial QIAamp DNA Blood Mini Kit on a QIAcube automated analyzer. SNPs rs1800469, rs1800470, and rs1800471 in the *TGFB1* gene were determined by real-time polymerase chain reaction using TaqMan probes on a CFX96 amplifier. **Results.** In children with BA, the occurrence of the investigated SNPs in *TGFB1* was as follows: rs1800469 – 38% GG homozygotes, 50% AG heterozygotes and 12% AA homozygotes; rs1800470 – 39% AA, 44% AG, 17% GG; rs1800471 – 88% CC, 12% GC, 0% GG. The distributions of all the three SNPs followed the Hardy–Weinberg principle. For rs1800469 and rs1800470, the genotype and allele frequencies in children with BA did not differ from those in healthy individuals, whereas for rs1800471, the heterozygous GC genotype was three-fold more frequent in children with BA than in healthy individuals. Haplotype analysis showed the presence of 6 major combinations: 2 most frequent were present in a total of about 66% of patients and 91% of healthy individuals, each of the frequencies practically did not differ between the comparison groups. Significant differences were found in the frequency of 3 rarer haplotypes, A-A-C, G-G-C and G-A-G at position rs1800469, rs1800470, rs1800471, which were observed more frequently in patients with BA by 3.10 (CI 1.59 to 6.04) (p = 0.001), 3.10 (CI 1.55 to 6.17) (p = 0.0015), and 17.02 (CI 1.94 to 149.30) (p = 0.011) times, respectively, than in healthy individuals. **Conclusion.** In children with BA, the occurrence of CG heterozygotes in rs1800471 and the distribution of three rare haplotypes A-A-C, G-G-C and G-A-G of the rs1800469, rs1800470 and rs1800471 SNPs in the *TGFB1* gene significantly differs from that in healthy individuals. It is possible that carriage of rare genotypes and haplotypes of *TGFB1* may predispose to BA in children.

Keywords: congenital and hereditary liver diseases, biliary atresia, pediatric liver recipients, liver transplantation, rs1800469, rs1800470, rs1800471, polymorphism.

INTRODUCTION

Pediatric liver transplantation (LT) is the only effective treatment for end-stage liver disease. Biliary atresia (BA) is the most common cause of liver failure in young children (up to 50% of cases) [1–3].

The incidence of BA, a rare congenital liver pathology, varies by country, ranging from 5 to 11 children per 100,000 newborns. The incidence is higher in Japan and China than in Europe, and girls are more likely than boys to have it [4, 5].

It is unclear exactly what causes BA. In addition to genetic considerations, environmental factors such as viral infection, toxins, or circulatory disorders during the fetal and/or prenatal periods are currently considered. The action of risk factors on the liver leads to inflammatory obliteration of the extrahepatic bile ducts and causes characteristic lesions in the intrahepatic biliary tree. Cholestasis, which results from damage to the common bile duct, triggers a cascade of other pathological processes, including parenchymal inflammation, bile acid buildup and toxic effects, cytokine activation that causes hepatocellular injury and dysfunction, development of fibrosis, which eventually progresses to liver cirrhosis [6–8].

Children with BA have significantly decreased levels of transforming growth factor beta-1 (*TGFB1*), an anti-inflammatory and profibrogenic cytokine that is believed to play a crucial role in the processes of inflammation and fibrosis. Although the exact mechanism regulating the *TGFB1* content in biliary atresia has not been investigated, genetic determination of the cytokine level due to protein polymorphism may be one of the key factors [9–11].

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The cytokine *TGFB1* may exhibit varying degrees of expression and activity in tissues due to the significant variability of the *TGFB1* gene. The *TGFB1* gene currently has three single nucleotide polymorphisms (SNPs) that are believed to be the most important: rs1800469 or C(–509)T – substitution of cytosine for thymine in the promoter region resulting in altered binding to transcription factors; rs1800470 or T(+869)C – substitution of thymine by cytosine in codon 10, leading to replacement of leucine by proline in the cytokine itself; and rs1800471 or C(+915)G – substitution of cytosine by guanine in codon 25, leading to replacement of arginine by proline in the protein [12, 13].

We have previously shown that children with end-stage liver disease have a significantly higher frequency of rare haplotypes of SNPs rs1800469, rs1800470, and rs1800471 in the *TGFB1* gene than healthy individuals. The causes of end-stage liver disease in the cohort under study included various congenital cholestatic or metabolic diseases, as well as acquired cirrhosis or hepatitis. This made it impossible to evaluate the role of the *TGFB1* genetic polymorphism in the development of a particular disease and necessitated the study of more homogeneous (in terms of causes of the disease) patient groups [14].

The aim of the present study was to evaluate the occurrence of the three SNPs in the *TGFB1* gene – rs1800469, rs1800470, rs1800471 – and their haplotypes in pediatric liver recipients with BA.

Evaluation of the role of the TGFB1 genetic polymorphism in biliary atresia would advance our knowledge of the pathogenesis of the disease and allow to come up with new personalized prognostic and therapeutic approaches to the treatment of liver recipients who have been diagnosed with this condition.

MATERIALS AND METHODS

The study included 106 pediatric liver recipients aged 4 to 150 (median, 8) months (44 boys and 62 girls) and 199 healthy individuals aged 32.7 ± 9.6 years (78 boys and 120 girls). The indication for pediatric LT was BA.

BA was diagnosed based on clinical, laboratory and instrumental studies. The examination included laboratory diagnostics, abdominal ultrasound scan and magnetic resonance cholangiopancreatography (MRCP). The activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (GGTP), alkaline phosphatase (ALP), bilirubin, albumin, total protein were determined. Biochemical parameters revealed signs of cholestasis – increased levels of transaminases, direct bilirubin, and alkaline phosphatase. Ultrasound evaluated the state of intrahepatic bile ducts. MRCP allowed to verify the diagnosis and to specify the anatomy of bile ducts. Morphological methods of investigation were performed at the pathological anatomy department of Shumakov National Medical Research Center of Transplantology and Artificial Organs (department headed by Dr. N.P. Mozheiko (MD)) and included macroscopic description and histologic examination of patients' liver samples.

All patients included in the study underwent living related LT, after which they received double or triple immunosuppressive therapy consisting of tacrolimus, corticosteroids and mycophenolate. Routine examination and treatment of patients were performed in accordance with the clinical guidelines of Shumakov National Medical Research Center of Transplantology and Artificial Organs.

Genomic DNA was isolated from peripheral blood using a commercial QIAamp DNA Blood Mini Kit on automatic analyzer QIAcube™ (Qiagen, Germany) according to the manufacturers' protocols. Polymorphic variants rs1800469 (G>A), rs1800470 (A>G), rs1800471 (G>C) of the TGFB1 gene were tested by real-time polymerase chain reaction using TaqMan probes (Applied Biosystems, USA) on a CFX96™ amplifier (Bio-Rad, USA) according to the manufacturer's instructions.

Statistical processing of the study results was performed using the Microsoft Excel program. Genotype distribution frequencies of the studied SNPs and haplotype structure were analyzed using the SNPstats program [15]. To confirm the independent distribution of alleles in the studied polymorphisms, their compliance with the Hardy–Weinberg principle was checked. The allele frequency was calculated as a percentage using the formula: Allele frequency = ((2 × number of homozygotes) + number of heterozygotes)) / 2 × total number of individuals. Pearson's chi-square test was used to compare the frequencies of genotypes or individual alleles in different groups. Odds ratios (OR) and their 95% confidence intervals (CI) were calculated to quantitatively represent the strength of influence of a possible genotype on a trait. The critical value of the significance level was taken as 0.05.

The protocol of this study was approved by the local ethics committee of Shumakov National Medical Research Center of Transplantology and Artificial Organs. Before participating in the study, patients or their guardians signed a written informed consent, which is kept in their medical records.

RESULTS

In pediatric liver recipients diagnosed with BA (hereinafter referred to as the "BA group") and in healthy adults (control group, hereinafter referred to as "healthy"), genotyping was performed and the occurrence of the 3 most significant TGFB1 gene SNPs – rs1800469, rs1800470, and rs1800471 – were analyzed (Fig.).

As can be seen in Fig., the frequencies of genotypes and alleles of two SNPs – rs1800469 (Fig., a) and rs1800470 (Fig., b) – in children with BA and in healthy individuals are virtually identical.

At the same time, significant differences were found in the distribution of rs1800471 genotypes (Fig., c): the

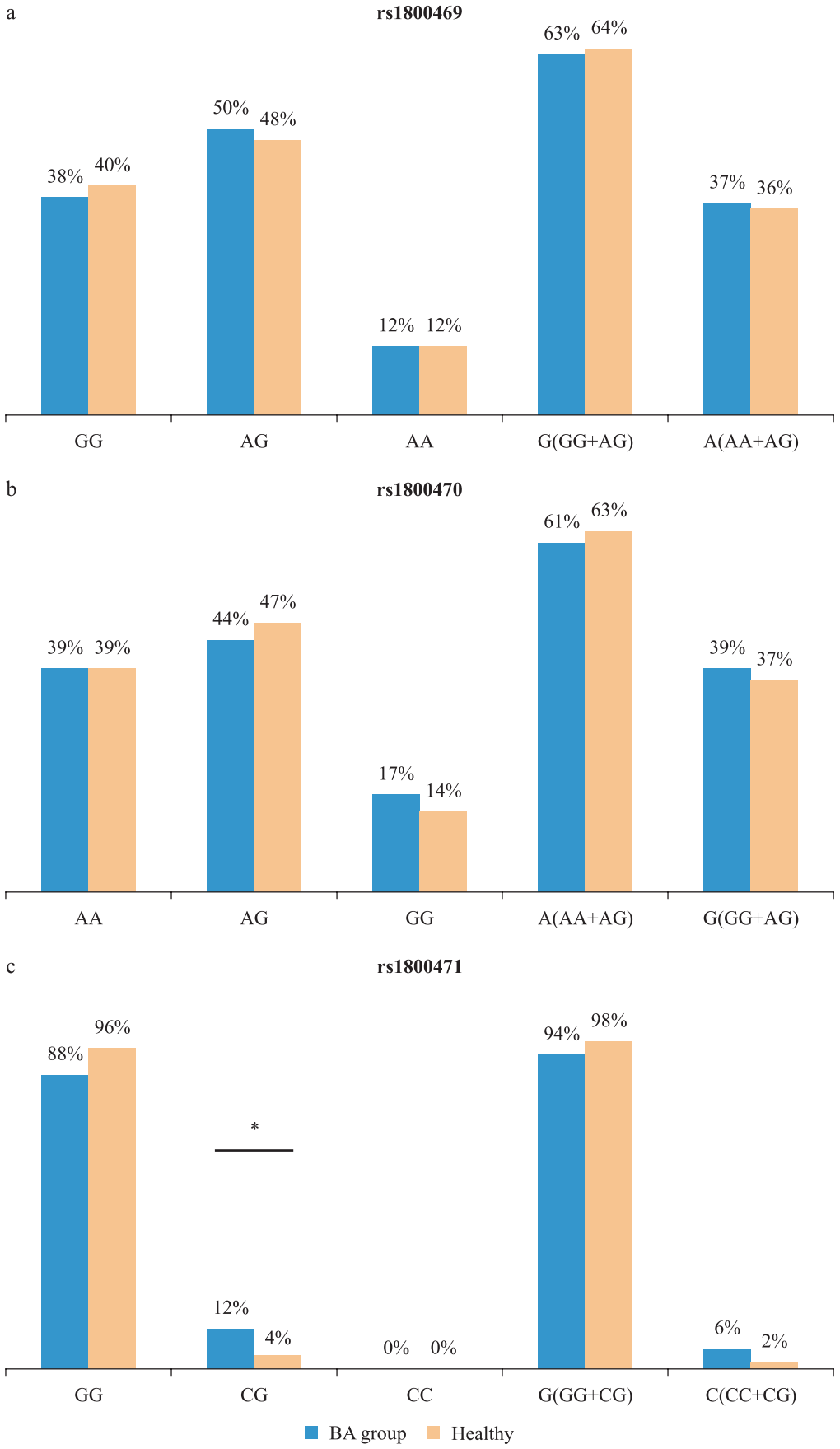

Fig. Distribution of genotypes and alleles of the TGFB1 gene SNPs – rs1800469 (a), rs1800470 (b) and rs1800471 (c) – in children with biliary atresia and in healthy individuals, * $p < 0.05$

heterozygous CG genotype was more than 3 times more frequent in children with BA than in healthy individuals (p = 0.014).

Analysis of allele distribution equilibrium in accordance with the Hardy–Weinberg principle revealed no significant deviations for any of the studied SNPs in both children with BA and in healthy individuals.

To assess the differences in the distribution of genotypes in children with BA and in healthy individuals in different allelic gene interaction models: codominant, dominant, recessive, and over dominant, genotype frequencies, odds ratios, and error bars were calculated for each SNPs (Table 1).

The results presented in Table 1 show that for SNPs rs1800469 and rs1800470, there are no significant differences in the frequency distribution of genetic variants in children with BA and in healthy individuals in any of the allelic gene interaction models.

For SNP rs1800471, there were significant differences in the distribution of genotypes in the only possible codominant model: the CG genotype was more frequent in children with BA than in healthy individuals, OR 3.15 (CI 1.24–7.97), p = 0.014.

The polymorphic areas being examined are found in a single gene, can be inherited in a linked manner [14] and form haplotype combinations that are stable in inheritance.

The distribution of haplotypes rs1800469, rs1800470, and rs1800471 in the study sample was analyzed using the SNPstats program. Table 2 shows the observed haplotypes in decreasing order of frequency of occurrence, frequencies for comparison groups, OR between patients and healthy controls, and the error value.

The result (Table 2) shows that there were 6 major combinations in the studied groups, two of which were the most frequent and were cumulatively present in 66% of children with BA and 91% of healthy individuals. Each of these frequencies did not differ significantly between the children and healthy individuals. The occurrence of haplotype #5, G-G-G, also did not differ significantly between the comparison groups.

At the same time, there were notable differences in the distribution of three rarer haplotypes among the groups under study: #3, #4, and #6 (G-G-C, A-A-C, and G-A-G). Haplotypes #3 and #4 were shown to be three times more prevalent in BA patients than in healthy in the individuals, whereas haplotype #6 was found to be 17 times more prevalent.

DISCUSSION

BA is a complex disease with an unclear etiology; it is likely caused by environmental and/or genetic risk factors. Genetic polymorphism of the key anti-

Table 1

Distribution of the TGFB1 polymorphism in children with biliary atresia and in healthy individuals in different allelic gene interaction models

SNPs/Model	Genotype	Frequency (% of BA)	Frequency (% of healthy)	OR (95% CI)	P value
rs1800469					
Codominant	GG	38.5	40.4	1.00	0.94
	AG	50.0	48	1.09 (0.66–1.82)	
	AA	11.5	11.6	1.04 (0.47–2.31)	
Dominant	GG	38.5	40.4	1.00	0.74
	AG-AA	61.5	59.6	1.08 (0.67–1.76)	
Recessive	GG-AG	88.5	88.4	1.00	0.98
	AA	11.5	11.6	0.99 (0.47–2.08)	
Over dominant	GG-AA	50.0	52.0	1.00	0.74
	AG	50.0	48.0	1.08 (0.67–1.74)	
rs1800470					
Codominant	AA	38.8	39.4	1.00	0.66
	AG	43.7	47.0	0.94 (0.56–1.59)	
	GG	17.5	13.6	1.30 (0.64–2.64)	
Dominant	AA	38.8	39.4	1.00	0.92
	AG-GG	61.2	60.6	1.02 (0.63–1.67)	
Recessive	AA-AG	82.5	86.4	1.00	0.38
	GG	17.5	13.6	1.34 (0.70–2.57)	
Over dominant	AA-GG	56.3	53.0	1.00	0.59
	AG	43.7	47.0	0.88 (0.54–1.41)	
rs1800471					
Codominant	CC	88.3	96.0	1.00	0.014*
	CG	11.7	4.0	**3.15 (1.24–7.97)**	

* p < 0.05.

Table 2

Distribution of the TGFB1 haplotypes in children with BA and in healthy individuals

S/N	Nucleotide in position			Frequency (%)		Odds ratio (95% CI)	P value
	rs1800469	rs1800470	rs1800471	BA	Healthy		
1	G	A	C	45.94	58.85	1.00	–
2	A	G	C	19.63	31.74	1.02 (0.67–1.57)	0.92
3	G	G	C	15.79	3.69	**3.10 (1.59–6.04)**	0.001*
4	A	A	C	12.76	3.72	**3.10 (1.55–6.17)**	0.0015*
5	G	G	G	4.15	1.75	2.31 (0.73–7.36)	0.16
6	G	A	G	1.73	0.25	**17.02 (1.94–149.30)**	0.011*

* $p < 0.05$.

inflammatory and profibrotic cytokine TGFB1 may be one of the causes.

The present study shows that in pediatric liver recipients diagnosed ITH biliary atresia, the frequency of polymorphic variants of the TGFB1 gene and its haplotypes differs significantly from that of healthy individuals.

The occurrence of SNPs rs1800469, rs1800470, and rs1800471 of the TGFB1 gene in healthy individuals, as reported in our work, is consistent with the Hardy–Weinberg principle and agrees with the findings by other authors and with the data presented in the NCBI database (US biotechnology information database) for the European population [16–18].

The distribution of the three *TGFB1* SNPs in children with BA also matches the Hardy–Weinberg equilibrium; the occurrence of genotypes and alleles rs1800469 and rs1800470 is the same as in healthy individuals, whereas the heterozygous CG genotype is three times more common in biliary atresia for rs1800471. The OR for the heterozygous CG genotype rs1800471 in children with BA in the codominant allelic gene interaction model averages 3.15 (CI 1.24–7.97) when compared to the healthy cohort, which may indicate a predisposition to the development of biliary atresia in those with this genotype. Given that there were only a few patients with the CG genotype in the study group, more research using a greater sample size is required.

Cytokine *TGFB1* is involved in the regulation of many key cellular processes, such as immune response, healing, apoptosis, and others; therefore, a significant impairment of its function may be incompatible with life [19]. It is possible that individual single nucleotide substitutions may have little effect on protein function, whereas a combination of several SNPs may be of clinical significance. As a result, we contrasted the haplotype frequencies of the three SNPs in children with BA and in healthy individuals.

The two most frequent haplotypes (G-A-C and A-G-C) were observed in 66% of children with BA and in 91% of healthy individuals, and the prevalence of each haplotype was not significantly different between the compared groups. Statistically significant differences were found in the distribution of 3 rare haplotypes (G-G-C, A-A-C, and G-A-G), which were generally found in 30% of children with BA and in 8% of healthy individuals. Children with BA were found to have rare haplotypes on average 3–17 times more often than healthy individuals, which may indicate that bearers of these haplotypes are predisposed to BA.

To date, no studies of the *TGFB1* genetic polymorphism in young children with BA in the Russian or other populations have been reported. In our previous work, we analyzed the distribution of *TGFB1* gene polymorphism in 225 children in the end-stage of liver failure as a result of various liver diseases, including BA [14]. The TGFB1 genetic polymorphism in young children with BA in the Russian or other populations has not yet been the subject of any published research. The distribution of *TGFB1* gene polymorphisms in 225 children with end-stage liver failure due to a variety of liver diseases, including BA, was examined in our earlier study.

Summarizing the results obtained in this study on the occurrence of polymorphic variants of the *TGFB1* gene and its haplotypes in children with BA and in healthy individuals, we can conclude that the *TGFB1* polymorphism plays a role in the development of BA in children. Therefore, the idea that genetic risk factors play a role in BA is supported by our findings.

The genetic hypothesis is supported by several facts such as familial cases of BA or data showing that 10–20% of children with BA also have other internal organ anomalies. In addition, full genome studies in Chinese and European patients with BA have identified the *XPNPEP1*, *ADD3*, and *PKD1L1* genes, presumably of functional significance for the development of BA. BA can now be regarded as a ciliopathy because of the intense interest generated in the past decade by data on multiple mutations in the genes that control the function of the ciliary apparatus (villi) of cholangiocytes in BA patients [7, 20, 21].

At the same time, it can be said that BA is a complex, heterogeneous disease that may be a common outcome of various disorders, given the abundance of data on its association with cytomegalovirus or human papillomavirus infection, as well as with the toxic effects of environmental factors during pregnancy [22].

CONCLUSION

BA, a rare congenital liver pathology, is most likely caused by environmental and/or genetic factors. The current study compared the distribution of the three most significant *TGFB1* polymorphisms – rs1800469, rs1800470 and rs1800471 – and its haplotypes in children with BA and in healthy individuals. Heterozygous CG genotype rs1800471 was shown to be three times more common in children with BA, while haplotypes G-G-C, A-A-C, and G-A-G (corresponding to rs1800469, rs1800470, and rs1800471) are three to seventeen times more common in these children than in the healthy cohort. This finding suggests that children carrying the CG genotype rs1800471 and haplotypes G-G-C, A-A-C or G-A-G corresponding to rs1800469, rs1800470 and rs1800471 of the TGFB1 gene may be at risk of developing BA.

The authors declare no conflict of interest.

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DEVELOPMENT OF A LOW PRIMING VOLUME HYDRODYNAMIC TEST BENCH FOR ISOLATED *EX VIVO* PERFUSION OF SMALL ANIMAL LUNGS

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Objective: to develop and validate a hydrodynamic test bench (HTB) with a small filling volume for *ex vivo* normothermic machine perfusion (NMP) of donor lungs of small experimental animals (rats) using the open-loop technique. **Materials and methods.** An HTB was developed for *ex vivo* NMP of donor lungs of rats. It is a prefabricated structure with stands that hold the following equipment: a ventilator for small laboratory animals, a heating element, a low priming volume membrane oxygenator and a dome for donor lung storage, as well as roller peristaltic pump, sensors and device for invasive pressure measurement in the circuit, bubble filter and a line kit. Wistar rats (n = 6) were used to investigate the effectiveness of the HTB. Following the removal of donor lungs, the graft was positioned on the HTB and *ex vivo* lung perfusion (EVLP) was initiated with selected parameters. During the rat donor lung perfusion procedure, *ex vivo* PaO_2/FiO_2 ratio, oxygenation index (OI), pulmonary artery pressure (PAP) and peripheral pulmonary vascular resistance (pPVR) were measured. **Results.** High OI values were obtained at the end of the procedure (460 ± 32 at p = 0.028); constant PAP values were recorded in all cases throughout the EVLP procedure – from 9.13 to 7.93 mmHg at p > 0.05. The criterion for HTB functionality was pPVR, which tended to decrease in all cases – from 603.3 ± 56 to 89.1 ± 15 dynes/sec/cm^{-5} at p = 0.000. No design flaws impacting the donor lungs' functional condition during *ex vivo* NMP procedure were found in the circuit of the hydrodynamic low priming volume bench during experimental studies. **Conclusion.** The efficiency and technical functionality of the HTB were demonstrated by the results of the experimental study conducted on the laboratory animals, rats. The observed dynamics of decrease in pPVR and the high OI values at stable PAP allowed for the conclusion that both the *ex vivo* perfusion itself and the technical design of the HTB are efficient.

Keywords: EVLP, ex vivo normothermic lung perfusion, hydrodynamic bench, low priming volume oxygenator, donor lung chamber.

INTRODUCTION

Introduction of *ex vivo* perfusion into clinical practice has made it possible to evaluate and rehabilitate initially compromised donor lungs and increase the number of transplants using suboptimal donor organs [1, 2]. Modern development of normothermic machine perfusion of donor lungs outside the donor body is extremely intensive and is aimed at improving lung transplant outcomes. According to world statistics, more than 30% of lung transplants initially deemed unsuitable for transplantation are successfully implanted in recipients after an *ex vivo* lung perfusion (EVLP) procedure, and 3-year patient survival is about 70% in the absence of chronic organ rejection [3, 4].

However, creation and introduction of new drugs and molecules, modified perfusion solutions and agents for cold cardioplegia of donor lungs, as well as active research on the use of mesenchymal stromal cells and gene products to reduce ischemia-reperfusion injury to the lung graft and the risk of severe primary dysfunction after implantation dictate the need to search for an optimal animal model for research [5, 6]. Although sheep and pig experimental models are the most common, economic limitations are a major obstacle to creating a large research sample [7–12]. In turn, today the rat model, which is characterized by a high degree of validity and reproducibility, has been actively introduced into biomedical research on the pathophysiology of EVLP and lung transplantation [13, 14]. Special attention should be paid specifically to a model for *ex vivo* normothermic perfusion of donor lungs of rats, since it is the perfusion procedure that allows a detailed comprehensive assessment of the graft and objectification of research results [15], but the lack of an established EVLP technique in small laboratory animals and the scientific search for an optimal perfusion protocol dictate the need to improve laboratory technologies for scientific research [16]. The use of modern 3D printing techniques, optimization of available equipment for laboratory animals and develop-

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ment of a universal EVLP protocol for rats will increase the efficiency of scientific research, and achieving a perfusion volume close to the volume of the animal's circulating blood will ensure the reliability of results when assessing the concentrations of biochemical markers and drugs [17].

A work was carried out to create a laboratory test bench with optimal functional characteristics for *ex vivo* normothermic perfusion of donor lungs in rats with a low priming volume circuit filling and integrated oxygenator [18, 19] to recreate optimal conditions for graft functioning outside the living organism.

In order to apply this innovation as a platform for preclinical research of new medications, the **objective** of this work is to perform EVLP in a rat model using a laboratory low priming volume bench to assess the performance of donor lungs during *ex vivo* perfusion.

MATERIALS AND METHODS

In this study, a complex hydrodynamic test bench (HTB) with a small circuit filling volume was developed for *ex vivo* normothermic machine perfusion of donor lungs of small laboratory animals (rats). Many components of the extracorporeal circuit were modeled, calculated and created based on the given medical and technical requirements corresponding to the size of the small laboratory animals. The basic scheme of the perfusion circuit device for EVLP, which took into account the existence of the main components for *ex vivo* procedure, served as a basis (Fig. 1).

The developed HTB is a prefabricated structure with racks on which the main components of the extracorporeal circuit are fixed (Fig. 2).

Prior to the first series of experimental investigations, perfusate was poured into the circuit, which circulated through the system and was heated to 37.0 °C. After the lungs were procured, they were placed in a specially designed container.

Computer-aided design software system SolidWorks (SolidWorks Corporation, Dassault Systèmes) was used to create this element of the system. The 3D mathematical model of the dome is shown in Fig. 3, a. The optimal

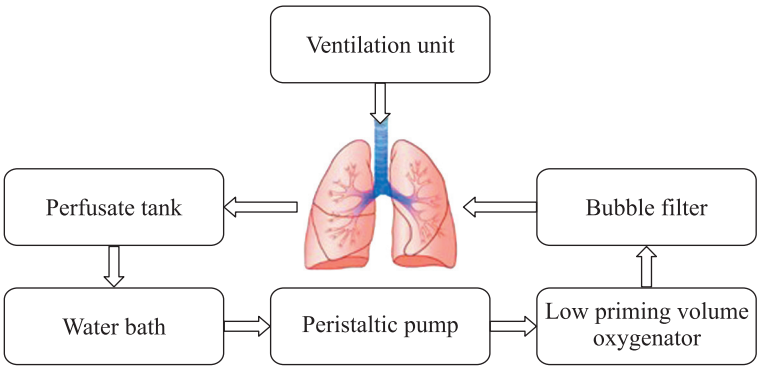

Fig. 1. Block diagram of the hydrodynamic test bench with low priming volume

Fig. 2. The developed bench for normothermic machine perfusion of lungs in small animals (1, ventilator; 2, heating device; 3, sealed container; 4, developed container for donor lungs; 5, roller (peristaltic) pump; 6, developed low priming volume membrane oxygenator; 7, pressure measuring apparatus; 8, bubble filter)

dimensions of the base with an opening for perfusate evacuation, the plate for placing the donor lungs of the animal and the container lid itself were all designed in this software environment and on the basis of topographic-anatomical studies on the rats (Fig. 3, b).

The container parts were created based on mathematical models (Fig. 4). Despite the small size (assembled: depth 30 mm, height 45 mm, diameter 65 mm), the design provided full functionality and efficiency.

Donor lungs were ventilated using a SAR-830/AP device (CWE, USA). The outlet of the organ container was connected to a flask. The sealed plastic container was placed in a heat-resistant laboratory beaker with distilled water and an immersed temperature sensor to control the temperature. The beaker was placed on a heating device (XMTE-205, China), which monitored the temperature of perfusate in the circuit.

The heat-exchange flask was connected in series with a Kamoer roller peristaltic pump (KCM-S403-ODMA, China). The speed range of this pump is from 0.1 to

a b

Fig. 3. The container for the donor lung of small animals: a, three-dimensional mathematical model of all components; b, topographic-anatomical location of the donor lungs in the chamber

350 rpm, which is suitable for the purposes of this study. Perfusate flow rate was reproduced within 1% at fixed rotational speed. After the pump, the perfused solution was passed through a specially designed membrane oxygenator with a low priming volume [11, 12]. This component was designed in a mathematical environment (Fig. 5, a). The inlet and outlet temperature perfusion streams at the selected design were analyzed in Ansys (ANSYS Inc., USA), a universal finite element analysis software system (Fig. 5, b).

The developed oxygenator was connected by a system of trunk lines to a bubble filter acting as an air trap in the system, and then the circuit was closed by a cannula entering the pulmonary artery of the donor lungs of the animals. The circuit also included ports for pressure measurement using a universal module based on the OEM IBP Angioton (Biosoft-M, Russia).

RESULTS

Male Wistar rats weighing 300–350 g (N = 6) were used for testing and evaluation of the functional characteristics of HTB. After heart-lung procurement and cold preservation of donor lungs, *ex vivo* normothermic perfusion was performed for 120 minutes, where the main parameters – oxygenation index (OI), pulmonary artery pressure (PAP) and peripheral vascular resistance in the lungs – were measured. During animal preparation and the donor lung procurement procedure, 20 ml dextran-40-based perfusion solution was poured into the circuit and circulation was switched on to complete a full circle and heat the perfusate. The system for monitoring *ex vivo* donor lung perfusion parameters continuously recorded the main parameters – PAP and volumetric flow rate – during two hours of the procedure. The heart-lung complex during the *ex vivo* normothermic perfusion procedure with dextran-40-based solution is presented in Fig. 6.

Fig. 4. Manufactured parts of the container for placing donor lungs of small animals

a b

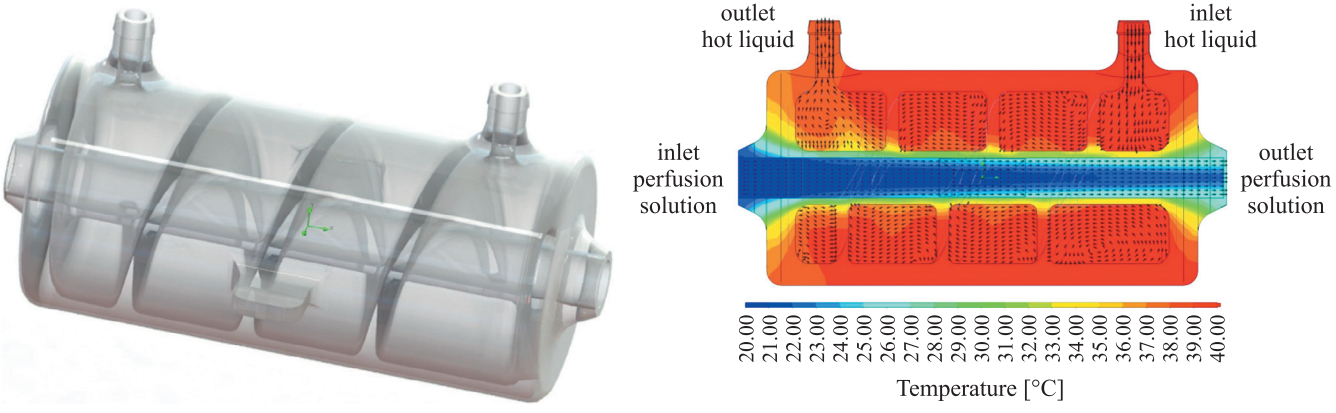

Fig. 5. Membrane low-volume oxygenator: a, three-dimensional model; b, analysis of temperature perfusion flows in the oxygenator design

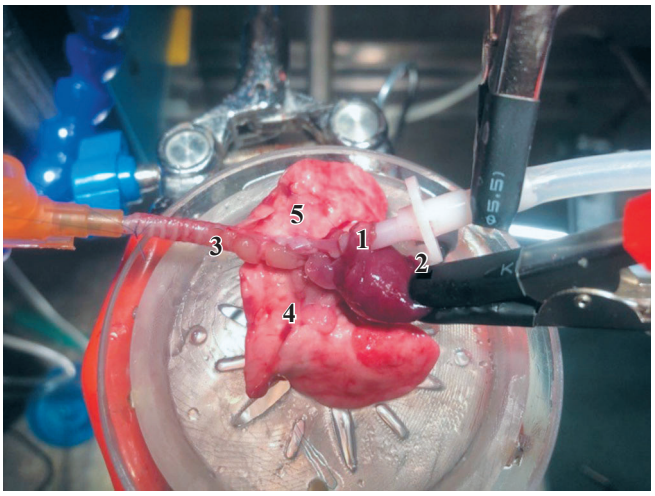

Fig. 6. Donor lungs and heart of an experimental animal (1, pulmonary artery; 2, left atrium; 3, trachea; 4, left lung; 5, right lung)

In a series of experiments to evaluate the functionality of the test bench during *ex vivo* normothermic perfusion of the lungs, the PaO_2/FiO_2 ratio, OI, PAP and peripheral pulmonary vascular resistance (pPVR) were measured. The measured results were entered into a data register, processed and summarized graphically (Fig. 7). All calculations were performed and compared by one-way analysis of variance (one-way ANOVA) using 95% confidence interval; the statistical significance of differences was considered reliable at $p < 0.05$.

OI was the main indicator of pulmonary function assessment and *ex vivo* perfusion efficiency that was investigated. The average OI at the beginning of *ex vivo* perfusion on the developed stand was 355 ± 20; in the course of the procedure, OI tended to increase, which by the end of the study averaged 460 ± 32 ($p = 0.028$). PAP constancy was the main criterion for the absence of technical problems during *ex vivo* donor lung perfusion. At the beginning of the procedure, PAP values averaged 9.07 ± 1.1 mmHg, and by the end of the study, where the mean value was 8.47 ± 0.4 mmHg, there was no statistically significant difference ($p > 0.05$). The pPVR was a calculated index demonstrating the compliance of the vascular bed, which suggests that both the *ex vivo* perfusion itself and the technical design of the bench are effective. Thus, pPVR decreased with time, where the initial mean value of the index was 603.3 ± 56 dynes·sec/cm^{-5}, and by the end of the procedure, mean pPVR decreased to 89.1 ± 15 dynes·sec/cm^{-5} at $p = 0.000$.

CONCLUSION

The design effort led to the development and testing of an experimental model of the HTB with a low priming volume for *ex vivo* normothermic machine perfusion of rat lungs. The obtained results demonstrate that functional and design features of the HTB not only maintain the initial functional status of the lung transplant, but also improve donor organ parameters. The presented data showed how effective and technically sound the developed HTB was.

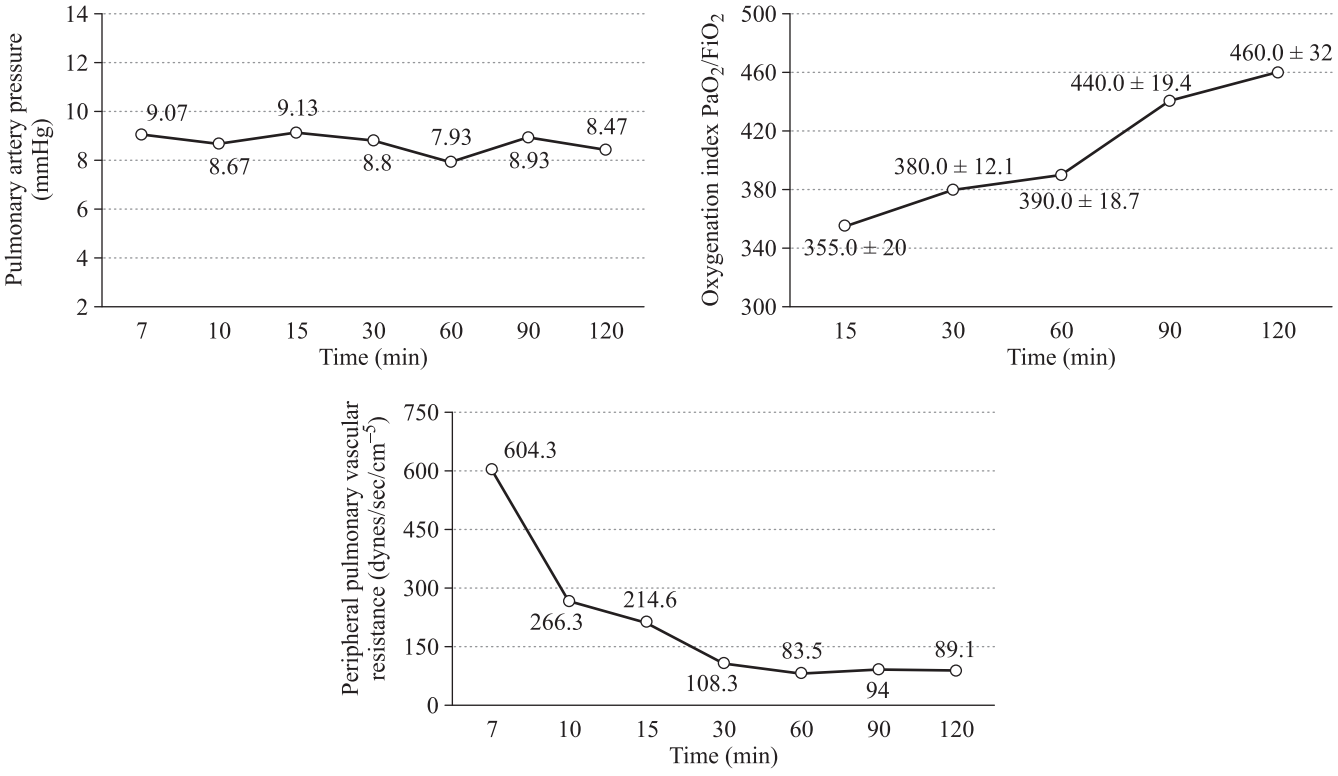

Fig. 7. Average values of parameters measured during experimental studies

The development of new techniques for examining the pathophysiological aspects of the impact of EVLP and lung transplantation on ischemia-reperfusion injury in donor organs is crucial for the development of new medications, perfusion, and preservative agents. One significant step in this process is the introduction and improvement of a HTB with a low priming volume for *ex vivo* normothermic perfusion of donor lungs on a rat model.

The authors declare no conflict of interest.

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LUNG TRANSPLANTATION MODELS FOR PRECLINICAL TRIAL (LITERATURE REVIEW)

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Lung transplantation (LT) is the only treatment for many end-stage lung diseases. Despite significant progress in transplantology and surgery, LT remains a high-tech surgical procedure performed at select research centers. Primary graft dysfunction, acute rejection, and chronic lung allograft dysfunction are serious problems that can worsen lung transplant outcomes significantly. Using animal models in experimental studies to investigate these pathologic conditions is one of the more rational approaches. A literature review was conducted in order to select a suitable model that reproduces pathologic processes developing after LT. The literature was searched and analyzed in MEDLINE and Elibrary databases, and the US National Institute of Health guidelines for the period up to December 2023 were reviewed. It was found that the most frequently used models are small laboratory animal models (without LT) and large animal models (with LT).

Keywords: lung transplantation model, primary graft dysfunction, acute lung rejection, chronic lung rejection.

INTRODUCTION

Lung transplantation (LT) is the only method of treatment for many end-stage lung diseases [7]. Although transplantology and surgery have advanced significantly, LT remains a high-tech surgical procedure performed at select research centers. In 2022, 14 lung transplants were performed in Russia [17]. Primary graft dysfunction, acute rejection, chronic lung allograft dysfunction are major problems that can significantly worsen LT outcomes. One of the rational approaches is to study these pathologic conditions in experimental animal studies [16, 26, 27, 37].In this context, summarizing the available data on the choice of an appropriate model that replicates the pathological processes that develop following LT appears to be useful.

A model that substantially approximates the clinical situation in preclinical studies of LT is one in which LT is reproduced, i.e., organ harvesting from a donor animal, preservation and surgical implantation into a recipient (Fig. 1) [4, 23, 24, 28, 40].

However, this model has significant drawbacks, such as extreme technical complexity (e.g., suturing mouse or rat pulmonary vessels) and high cost (operating microscope and/or use of a heart-lung machine and oxygenators for dual pulmonary transplantation) [4, 15, 24, 28, 30].

It should also be noted that the surgical technique of LT in experiments on large and small animals differs significantly.

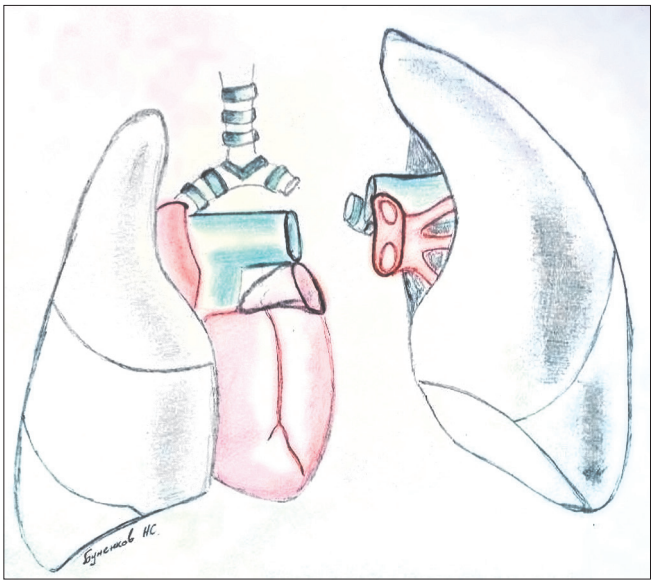

Fig. 1. Simplified diagram of single lung transplantation

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We were able to gather data on the best models for LT preclinical investigations by analyzing the MEDLINE database, searching for publications in the Russian National Library and the scientific electronic library eLibrary from 2013 to December 2023.

The following models are used to study LT outcomes in the experiment:

1. Primary graft dysfunction:
   a. hilar occlusion;
   b. *ex vivo* perfusion of isolated lungs;
   c. working with cell cultures.
2. Acute rejection:
   a. heterotopic tracheal transplantation;
   b. orthotopic tracheal transplantation;
   c. orthotopic lung transplantation.
3. Chronic rejection:
   a. heterotopic tracheal transplantation;
   b. orthotopic tracheal transplantation;
   c. intrapulmonary tracheal transplantation;
   d. bone marrow transplantation;
   e. orthotopic lung transplantation.

Primary graft dysfunction (PGD) is an acute lung allograft injury of varying severity, ranging from mild capillary leak in alveoli to severe diffuse alveolar damage occurring within the first 72 hours after LT. PGD is characterized by radiographic evidence of pulmonary edema and a progressive hypoxemia for no apparent reason [12, 30].

***In vitro* modeling of PGD** involves exposure of relevant pulmonary cells in cultures (or cocultures) to acute hypoxia followed by reoxygenation [5, 10, 41, 43]. A model for storing cell cultures in a preservation solution (Perfadex) at 4 °C before allowing the cells to warm to room temperature, followed by reoxygenation in 37 °C culture media is also characterized. *In vitro* models have demonstrated that longer cold aerobic times enhance apoptosis, cytoskeletal remodeling, permeability, as well as upregulation of innate and adaptive immune pathways. *In vitro* studies allow rational use of resources, but they must be validated by *in vivo* experiments [6, 30, 51].

***In vivo* modeling of PGD** is possible using unilateral hilar occlusion followed by reperfusion (Fig. 2).

Under mechanical ventilation, a thoracotomy is performed, and a clamp or ligature is applied to the hilum. This model is widely used in small rodents [30, 35]. The sham-operated group includes animals with access to the hilum without clamping it. The disadvantage of this model is that it requires attention to the mechanical ventilation aspect, as this may lead to ventilation-induced lung injury that can add to ischemia-reperfusion injury (IRI). However, using the combination of ventilation-induced injury and IRI may parallel what can occur during human lung transplantation IRI [30, 50].

Another variation of the hilar clamp model involves isolating and clamping the pulmonary artery alone to preserve gas exchange, and is referred to as the non-hypoxic lung ischemia model [30].

Additionally, IRI has been studied using isolated, perfused rodent lung models in which the lungs are manipulated either *ex vivo* or *in situ*, and continuously perfused with synthetic media and ventilated in a temperature-controlled chamber [14, 18, 19, 30, 38].

Orthotopic single lung, autologous or allogeneic LT is also used to study PGD. In this approach, the cold ischemic time of the donor lung is intentionally prolonged up to 18 hours [25, 30]. In the case of allogeneic transplantation, in addition to PGD, the model allows studying antigen-independent processes preceding acute rejection.

The key criteria for evaluating the outcome of preservation are: 1) determination of the degree of lung edema (weighing the organ before/after reperfusion); 2) translocation of exogenously administered Evan's blue dye or radiolabeled or fluorescently labeled proteins, or by measuring the accumulation of endogenous proteins (total protein, albumin, IgM) in the bronchoalveolar lavage fluid [43]. Testing for cardiopulmonary hemodynamics, lung function, and oxygenation using indwelling devices, surface probes, and arterial blood gases can also be performed [29].

Lung IRI is accompanied by lipid peroxidation and has been associated with reduced arterial oxygenation, decreased compliance and increased pulmonary vascular resistance [30]. Fibrin deposition, elevated expression of plasminogen activator inhibitor-1 (PAI-1), extravasation and recruitment of immune cells, increased levels

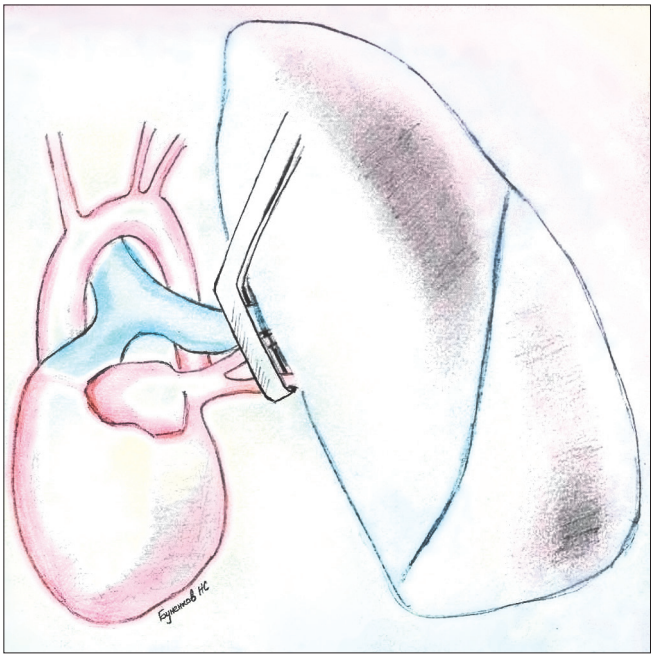

Fig. 2. Hilar occlusion is one way to study pathophysiologic changes after lung transplantation

of alarmins (particularly HMGB1), myeloperoxidase (a marker of neutrophil/mononuclear phagocyte activation and infiltration), and proinflammatory cytokines and chemokines are also noted in the lungs [29, 30, 39].

An advantage of the hilar transplant model is that it is less technically demanding than the single-lung transplant model. The model is a suitable platform for testing therapeutic delivery, lung rehabilitative potential, and biomarkers predictive of PG.

The orthotopic LT model is more informative when surgical technique needs to be developed and for studying post-transplant immune responses [30, 47].

The technique of allograft optimization through *ex vivo* lung perfusion (EVLP) has generated significant interest [19, 30, 38]. Most models using EVLP have utilized human lungs or large animal (mainly porcine) models with or without subsequent transplantation [30].

Imaging in PGD. Solid organ transplantation is accompanied by an immune response due to the presence of foreign antigens. Methods to image mobilization and activation of the immune system during this period are currently limited. However, SPECT (single-photon emission computed tomography) and PET (positron emission tomography) can detect and monitor a variety of pathophysiological processes, such as T cell activity by glucose uptake and neutrophil activation by binding to the formyl peptide receptor 1 (FPR1). The use of multiphoton intravital microscopy for imaging the PGD has also been reported [30].

Modeling of acute rejection. There are two forms of acute rejection (AR): acute cellular rejection (ACR) and antibody-mediated rejection (AMR) [30]. ACR is well characterized and is classified into two subtypes: 1) type A is a lymphocytic inflammatory cellular infiltrate that ranges from a mild perivascular infiltrate with no obvious tissue injury, to infiltrates that also involve the interstitium and air-spaces with prominent acute lung injury (ALI) with vasculitis; type B airway inflammation, namely lymphocytic bronchitis, is currently classified as either low-grade, with no tissue injury, or high-grade, in which there is a more extensive infiltrate associated with injury to the airway [3, 30]. Both A and B types of ACR are thought to increase the risk of development of bronchiolitis obliterans. The mechanisms of AMR are not as detailed, but are known to include C4d deposition in capillaries, neutrophilic capillaritis, intravascular macrophages, and ALI. Preclinical models are suitable for studying both ACR and AMR [30, 36].

Acute lung rejection in rats and mice. Since the 1960s, experiments using canine and rat orthotopic lung transplant models have examined various aspects of AR [30, 36]. For example, in a rat model, the onset of AR was more rapid in lungs when compared with heart grafts. The most commonly used mouse model to study lung rejection in the 1990s and early 2000s was heterotopic tracheal transplantation. In this model, allograft rejection demonstrates early inflammation, epithelial necrosis, and fibroproliferation in the airway lumen, which were not observed in the isografts. This model is valuable for studying ACR as well as chronic rejection.

Later, the orthotopic tracheal transplantation model was developed to study early changes in AR. The intrapulmonary tracheal transplant model as a model of AR has been useful to show the importance of intrapulmonary *de novo* lymphoid tissue [30].

Orthotopic LT in mice, while highly technically challenging, allows to observe histological changes as early as 3 days after transplantation from an MHC-incompatible donor, which are accompanied by perivascular and peribronchial mononuclear infiltration. These changes are similar to those observed in transbronchial biopsies of lung transplant patients suffering from AR [8, 30, 34]. The mouse model of orthotopic LT allows for the design of experiments to evaluate the role of respiratory pathogens on alloimmunity and AR responses. Pseudomonas aeruginosa respiratory infections can break immunosuppression-mediated lung allograft tolerance. This model is a suitable platform to evaluate new diagnostic modalities for AR. For example, fluorodeoxyglucose PET (FDG-PET) can be used to monitor acute lung allograft rejection owing to a high rate of metabolism of graft-infiltrating T cells [30]. Thus, the orthotopic mouse lung transplant model is an effective experimental platform to study mechanisms that contribute to AR, test noninvasive diagnostic modalities as well as to study immune tolerance, and evaluate strategies to prevent or treat this complication.

Antibody-mediated rejection. Elicitation of immune responses to the mismatched donor human leukocyte antigen (HLA) and breakdown of tolerance to tissue-restricted self-antigens pose a significant challenge to acceptance and continued graft function following organ transplantation. While the mechanisms of AMR are not firmly established, *de novo* donor-specific antibodies against HLA have been shown to predispose to the development of immune responses to lung self-antigens and bronchiolitis obliterans syndrome [22, 30]. To define mechanisms leading to anti-MHC–mediated development of rejection, a preclinical murine model was developed in which exogenous anti-MHC was administered into the native lungs and elicited production of antibodies and T cell responses specific for lung-associated self-antigens, type V collagen [col(V)], and K-α 1 tubulin, culminating in fibrotic pathology [22, 30]. Human lung transplant recipients develop antibodies against col(V), a protein predominantly found in the interstitium and not ordinarily exposed to the immune system [30]. In the rat LT model, allografts in minor histocompatibility

complex–mismatched recipients induce col(V)-specific T and B immunity after transplantation and appear to be an important source of autoantigen that autoantibodies ligate [30]. To study the exact roles of allo- and autoantibodies in acute and chronic rejection, orthotopic LT mouse models are more preferable [30, 33].

Microvascular injury and large airway disease. Autopsy results of patients who died with bronchiolitis obliterans syndrome showed that chronic rejection correlated with microvascular injury of the airway [30]. Such a relationship between microvascular destruction during AR and subsequent chronic rejection has been suggested with all solid-organ transplants.

The orthotopic tracheal transplantation (OTT) model is useful for the study of airway microvessels because the tracheal vasculature can be easily visualized in one tissue plane [9, 30, 44]. Tracheal transplantation is performed by simply cutting off the donor trachea and suturing it into the recipient in place of the excised section of the native trachea; alternatively, the donor trachea can be sewn in parallel to the native airway. During AR, this model has revealed that the airways undergo a transient loss of a functional microcirculation accompanied by localized tissue hypoxia and ischemia; although a functional microcirculation returns, these grafts cannot be rescued with immunosuppression once the vascular bed is transiently lost [30, 33]. Thus, the development of chronic rejection is also based on non-immune mechanisms. The proposed OTT model can incorporate fiber optic bioprobes that detect tissue oxygenation and perfusion over time. Another facet of a compromised circulation occurs at the time of transplantation in the airway anastomosis that does not include a restored bronchial circulation and is susceptible to dehiscence and infectio. The relative ischemia of the airway anastomosis is associated with a proclivity to infection, especially with *Aspergillus* and *Pseudomonas* [30].

The tracheal allograft model is a recognized and effective platform for preclinical studies, but it is less relevant nowadays because in clinical practice it can be decellularized and repopulated with recipient-derived cells prior to surgical implantation, an approach that should prevent AR and limit the need for chronic immunosuppression [1, 20, 30]. A more limited repopulation of donor-derived cells can be observed *in vivo* using the mouse OTT model. This line of study may have value in determining how both destructive and reparative processes occur through the migration of cell populations. OTT also facilitates lineage fate mapping studies to track the movement and transformation of various cell types in the allograft recipient. The mouse orthotopic lung transplant model also holds promise as an effective platform for evaluating the relative contribution of recipient cells in both the disease and repair of small airways and pulmonary parenchyma [30].

Lymphatic contribution to acute rejection. In a healthy lung, there is a highly complex network of lymphatics consisting of subpleural lymphatics largely over the lower lobes, and a deeper lymphatic network running along the major airways and the blood vasculature in the interstitial spaces [30, 45]. The visceral pleura and the neighboring lung tissue are drained through the superficial network into the hilar area of the lung where they connect with the deeper plexus of lymphatics. At the time of transplant, the bronchus, bronchial artery, pulmonary artery, and vein are severed at the level of the hilum. However, only the bronchus, pulmonary artery, and vein are re-anastomosed. A recent clinical study has revealed that, unlike chronic organ failure in kidney transplantation, lymphangiogenesis is not altered in patients with chronic lung allograft dysfunction [48]. In a canine lung transplant model, functional lymphatic drainage was restored at seven days after transplantation in isografts [30]. In allografts treated with immunosuppression, a functional lymphatic bed is observed between 2 and 4 weeks after transplantation. Lymphatic biology can also be effectively studied in the mouse orthotopic lung transplantation model, which revealed a marked decline in the density of lymphatic vessels, accompanied by accumulation of low-molecular-weight hyaluronan in mouse orthotopic allografts undergoing AR [34]. Work in this model has suggested a protective role for the promotion of lymphangiogenesis in the posttransplant period. In general, the role of the lymphatic vessels in acute and chronic rejection in lung transplants is poorly understood and requires more serious research.

CHRONIC LUNG ALLOGRAFT DYSFUNCTION (CLAD)

A high rate of chronic graft failure continues to be the most significant hurdle in improving long-term survival after lung transplant. In the 1980s, obliterative bronchiolitis (OB) was identified as a common pathology in chronically failing lung transplants; [30, 48]. OB was subsequently discovered to also be a complication of bone marrow transplant recipients. Histologic features of lung transplant–associated OB include anatomic restriction to membranous and respiratory bronchioles, vasculopathy with progressive myointimal thickening of the pulmonary arteries and veins [48]. Clinically, OB presents as a progressive obstructive decline in lung function termed bronchiolitis obliterans syndrome (BOS) [45, 48]. Approximately 50% of patients demonstrate this syndrome by five years after transplant [21, 30]. Clinical studies demonstrate a strong link between AR, specifically airway involvement with lymphocytic bronchitis, and BOS [13, 30].Other complications in the

posttransplant period such as PGD have also been linked to BOS. While BOS remains the predominant cause of CLAD, more recently a restrictive allograft syndrome (RAS) has been described. Patients presenting with RAS have been demonstrated to exhibit distinct histologic phenotypes such as pleural and subpleural fibrotic changes, intra-alveolar fibrinous exudate, and acute fibrinous pneumonia [30, 48]. A recent model has been developed utilizing fully MHC-mismatched orthotopic lung transplants treated with chronic immunosuppression and evaluated ten weeks after transplant; these mice develop some features of RAS [30].

***In vitro* modeling of obliterative bronchiolitis** involves the use of individual cell populations, such as bronchial epithelial cells, mesenchymal stromal cells, and airway smooth muscle cells [30]. Animal models involve allogeneic tissue transplantation to reproduce fibrotic airway remodeling by heterotopic or orthotopic transplantation of the trachea, as well as orthotopic transplantation of a single lung [30, 40].

Tracheal transplant models of chronic rejection. Initial discoveries in posttransplantation OB have been fueled primarily by the heterotopic tracheal transplantation (HTT) model in which a harvested donor trachea is transplanted into a subcutaneous pouch in the dorsal surface of the neck or omentum. [30, 46]. Following the IRI and AR phases of injury, these transplants undergo a fibroproliferative phase with partial denuding of the epithelium at day 14 and fibro-obliteration of the allograft trachea at day 21 [30, 46]. Conversely, the isografts have a healing airway graft at day 7; however, this is followed by essentially normal isografts by days 14 and 21 [30]. This model has been utilized in both rats and mice, although more consistent fibrosis is noted in rat versus mouse tracheas and is suitable for studying alloantigen-mediated airway fibrosis. Airway luminal obliteration can be quantified at various time points after transplantation. Fibrosis can be evaluated by staining with picrosirius or trichrome. In addition, the trachea can be treated with enzymes followed by flow cytometry of the cell suspension to assess cellular composition. Also, this model is convenient for evaluation of the microvasculature and lymphatics, providing an avenue to study their role in OB development.

The major criticism of the tracheal transplantation model is that fibrotic obliteration is being modeled in a large cartilaginous airway that is histologically distinct from the small airways that are the site of human OB [30, 49]. Its relevance is also somewhat limited by the absence of a normal air interface and native mediastinal lymphatic drainage. Most importantly, human OB develops in a complex *in vivo* environment with distinct cellular niches that cannot be reproduced in a tracheal transplant placed in an extrapulmonary environment [30, 42]. Thus, the HTT model is useful as a high-throughput screen for alloimmunity-induced fibrosis, but findings obtained with this procedure must be interpreted with caution [30, 49].

In the OTT model, epithelial regeneration from migration of recipient-derived epithelial cells limits the development of fibrotic occlusion or OB. Although obliterative lesions are not observed, OTTs develop lymphocytic bronchitis (a large airway precursor of BOS) as well as subepithelial fibrosis [11, 30].

The intrapulmonary tracheal transplant model via thoracotomy has also been used to attempt to simulate airway fibrosis. Another good model involves transplantation of human trachea together with peripheral blood mononuclear cells into an immunodeficient mouse [30].

Orthotopic lung transplant models of chronic rejection. Orthotopic single-lung transplantation in rats is successfully used to study acute rejection. However, it significantly limits the possibilities of modeling chronic rejection, because it does not allow to induce OB. LT across MHC mismatch and across minor histocompatibility complex–mismatched combinations has been shown to recapitulate some aspects of OB pathology in allografts. However, there is some disagreement in the transplant community about how closely OB-like lesions generated in orthotopic lung transplants recapitulate the human lesion [30, 42]. Other donor-recipient combinations have also been demonstrated to develop OB-like lesions at late time points of 2 to 3 months after transplantation. However, there appears to be a difference in animals obtained from different vendors as well as concerns of reproducibility across centers [30, 49]. Therefore, at present, a consensus on a definitive rat lung transplant model to study OB pathogenesis has not emerged [30, 42]. Attempts to approximate the rat model to the clinical situation via intratracheal gastric fluid challenge after allogeneic LT have been reported [31].

The use of MHC-mismatched mice allows to obtain severe AR by day 7 after LT, and this nearly complete destruction of the lung prevents longer time-point evaluation for development of OB, so that the animal dies before it develops OB [30, 49]. This issue was circumvented and a transplant involving minor histocompatibility complex mismatch developed only mild rejection within 1 week. Peribronchial and intraluminal fibrotic lesions were described at days 21 and 28 [30]. However, these airway fibrotic lesions were noted in only 50% of the transplanted mice and were limited to a small number of airways in the allografts [31]. Use of immunosuppression (cyclosporine + steroids) to prolong graft life in MHC mismatch has also allowed for investigation of the development of OB pathology. This model only generates OB-like lesions in 25–50% of the mice, with many mice demonstrating no evidence of OB or regaining normal histology after 12 weeks; the remaining

animals showed no OB signs or a histological picture of a healthy lung [30, 49].

Fibrotic remodeling of the allograft is the predominant cause of CLAD; hence, a relevant animal model for investigating CLAD must recapitulate allograft fibrogenesis and allow for meaningful targeting of specific pathways. Mesenchymal cells act as the first link in the development of fibrosis [30, 32]. These donor graft-derived cells appear to be the predominant contributors to OB lesions [30, 32]. In a heterotopic tracheal transplantation model, mesenchymal cells contributing to fibrosis originated from recipient cells [30, 49]. In an allogeneic orthotopic LT model in mice, it was shown that collagen I–expressing cells were of donor origin [30]. Thus, in the study of the role of mesenchymal cells in chronic rejection, the whole-lung transplant model holds an advantage over tracheal transplantation because it is more reflective of the clinical situation [30, 32]. The choice of models for studying the mechanisms of post-LT rejection is presented as a scheme in Fig. 3.

ASSESSMENT OF THE DEGREE OF GRAFT INJURY IN EXPERIMENTAL MODELS

The American Thoracic Society in 2022 released an official document with guidelines for the assessment of ALI in animal experiments [29]. The document is based on the results of a survey of 50 experts working in the field of clinical (pulmonology, intensive care, pediatrics, immunology, etc.) and basic medicine (cell biology, physiology) studying ALI [29].

In clinical practice, ALI is described using the term "acute respiratory distress syndrome (ARDS)", but small animal models lack some of the ARDS manifestations and therefore cannot fully reproduce the clinical situation [2, 29]. Large animals are more likely to reproduce the clinical manifestations of ARDS, so the results of experiments in large animals are important for clinical application [29].

The American Thoracic Society recommends the use of an ALI model that demonstrates the following four domains: 1) histological evidence of tissue injury, 2) alteration of the alveolar–capillary barrier, 3) presence of an inflammatory response, and 4) physiologic dysfunction (Table) [29].

According to the American Thoracic Society guidelines, a preclinical model of acute lung injury should demonstrate at least three of the above four domains for lung injury [29]. It was proposed that demonstrating ALI requires measurement of at least one parameter for each of the four domains. In the case of preclinical drug testing or translation into clinical practice, demonstration of all four ALI domains with the presentation of at least one indicator for each domain is recommended [29].

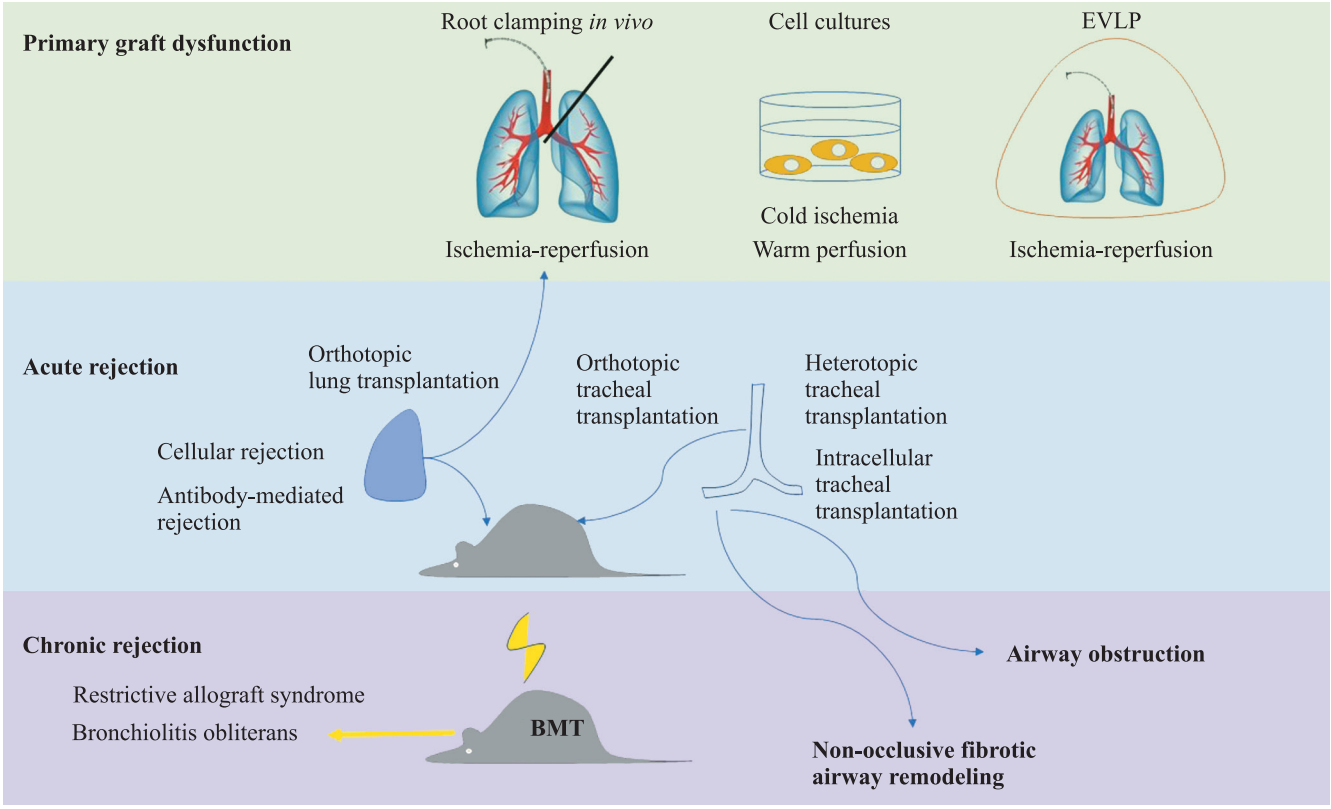

Fig. 3. Modeling of pathological processes after lung transplantation. Note: BMT, bone marrow transplantation; EVLP, *ex vivo* lung perfusion

Table

Parameters reflecting the degree of lung injury in animal experiments

Domains	Domain recommendations
Histological evidence of tissue injury	– Pulmonary alveolar proteinosis – Evidence of alveolar epithelial injury (cell death, epithelial denudation, ATII proliferation) – Neutrophil infiltration of the alveolar space – Thickening of alveolar septae and/or interstitial edema – Diffuse alveolar damage pattern – Respiratory distress syndrome – RBCs in the airways or extravasated red cells – Neutrophil infiltration of alveolar septae or interstitium – Perivascular inflammation, including intravascular accumulation of neutrophils – Perivascular edema – Hepatization – Weakening or loss of tight intercellular junctions – Presence of microthrombi
Alteration of the alveolar-capillary barrier	– Elevated BAL albumin, IgM, or other large circulating protein – Increased lung wet-to-dry weight ratio – Elevated BAL total protein – Evan's blue dye accumulation in lung homogenate – Increased Pulmonary vascular permeability index and/or filtration coefficient – Rate of accumulation of tagged marker (fluorescent probe, I-131 albumin, etc.) in the airspace – Transport of large-molecular-weight substance (≥70 kD, e.g. Dextran) – Accumulation of airspace-injected tracers into the circulation – Circulating markers of epithelial and/or airway injury (RAGE, SP-D, KL-6) – Hemorrhage and/or RBCs in airspace – Elevated BAL RAGE – Transport of a very large (~300 kD) tracer across barrier – Decreased surfactant function
Presence of an inflammatory response	– Increase in chemokines or cytokines in BAL or lung tissue – Increased neutrophil count in BAL or lung tissue (absolute count or by neutrophil elastase or myeloperoxidase content) – Increased pro-inflammatory monocyte and macrophage (and/or lymphocytes) subpopulations in BAL or lung tissue – Endothelial cell adhesion molecule expression or mediator release – Changes in acute response genes – Inflammasome activation – Mitochondrial dysfunction – Neutrophil extracellular traps
Evidence of physiological dysfunction	– Arterial blood gas measurements of oxygenation – Decreased compliance (distensibility) – Changes in alveolar fluid clearance – Decreased oxygenation measured by non-invasive methods – Respiratory deterioration – Lung changes in lung imaging – Dead space and/or partial pressure of carbon dioxide – Weight loss – Systemic organ dysfunction – Impaired systemic hemodynamics

Note: ATII, Type II alveolar epithelial cell; RBCs, red blood cells; KL-6, Krebs von den Lungen-6; RAGE, receptors for advanced glycation end products; SP-D, surfactant protein D; BAL, bronchoalveolar lavage; MCP, monocyte chemotactic protein.

The term "acute injury" implies the establishment of a certain time frame from the moment of exposure to the damaging factor to the manifestation of the above-described injury signs. However, at present, the exact time interval is not defined [29, 45]. Some authors indicate that an injury should be considered acute if the signs of injury occurred within 24 hours after exposure to an unfavorable factor [29]. Others accept 72 hours, up to 7 days, and even up to 10 days [29]. Thus, according to the American Thoracic Society guidelines, the time interval can be chosen depending on the goals of the study, believing that an interval of 24 hours is too strict [29].

CONCLUSION

In vitro and *in vivo* models may be recommended for studying PGD, specifically hilar transection surgery without LT, or orthotopic LT. The use of EVLP is also possible.

An HTT model and the more technically demanding OTT are recommended for studying acute rejection. Orthotopic LT is also suitable for investigating immunological tolerance.

Obliterative bronchiolitis in terminal bronchioles can be modeled in experiments with HTT. OTT allows the study of large airway lesions in chronic rejection, but occlusive lesions are not observed. Orthotopic allogeneic LT in mice is an extremely technically demanding model for studying chronic rejection, and is characterized by instability and heterogeneity in the results obtained. It should also be noted that the need for a model that reproduces restrictive allograft syndrome has not yet been met.

At least three or four criteria are used in experimental models to determine the severity of graft injury after LT, including histological evidence of tissue injury, alteration of the alveolar–capillary barrier, inflammatory response, and physiologic dysfunction. It is necessary to continue developing models that replicate the pathogenic processes that patients experience following LT.

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